8EHQ0703-14514

SOLVAY AMERICA

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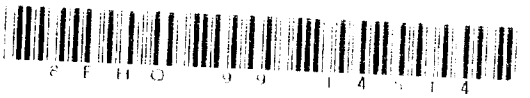

Re: Solvay Fluorides---TSCA Section 8(e)---Nocolok Flux—Follow-up to Number 8EHQ-0799-14514

To Whom It May Concern:

Summary

Enclosed is a study that is a follow-up to an earlier submittal for TSCA 8(e) made in 1999. The conclusions of the author of this latest 28 day inhalation study supports those made in the original 28 day study but at lower concentrations of exposure.

Background

This letter is being submitted by Solvay Fluorides, Inc. ("Solvay Fluorides") pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA).

On July 19, 1999, Solvay Fluorides submitted a TSCA 8e (**Number 8EHQ-0799-14514**) reporting a sub-chronic (28 day) inhalation toxicity study with Nocolok® flux in rats conducted in June, 1999.

On March 10, 2003, Solvay Fluorides reported to EPA preliminary information from a similar type 28 inhalation study recently conducted at lower concentrations than the first study (the lowest concentration studied was 1mg/m3).

In this transmission, we are including the actual report mentioned in our March 10th correspondence. It is entitled "A sub-acute (28 day) inhalation study with NocolokTM flux in male Wistar rats." It was produced by TNO in the Netherlands.

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Solvay America, Inc.
3333 Richmond Avenue, Houston, Texas 77098-3099 713/525-6000 Fax: 713/525-7887
Solvay Companies Website: http://www.solvay.com

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The author's conclusion for this study supports the findings of the first study but at lower concentrations of exposure. The conclusion made by the author was that the Minimal-Observed-Adverse-Effect Level was considered to be 1 mg/m3.

Please feel free to contact us if you need additional information.

Very truly yours,

Lisa A. Sherrill

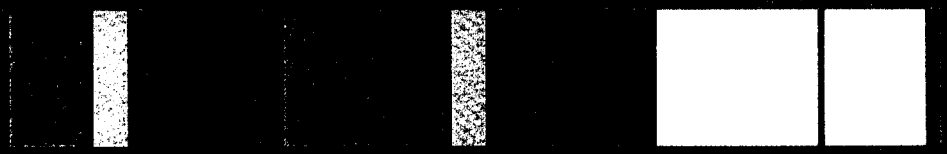
Lisa A. Sherrill
Attorney

Enclosure

TNO-rapport / TNO report

V 4671/01

A sub-acute (28-day) inhalation toxicity study wi NOCOLOK™ flux in male Wistar rats



Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek / Netherlands Organisation for Applied Scientific Research

TNO Nutrition and Food Research

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TNO Report

V 4671/01

A sub-acute (28-day) inhalation toxicity study with NOCOLOK™ flux in male Wistar rats

Date	22 May 2003
Authors	Dr Ir J.H.E. Arts Dr C.F. Kuper
Sponsor	Solvay SA Ransbeekstraat 301 1120-Bruxelles, Belgium
TNO project number	44152
TNO study code	4671/01
Sponsor's study code	
Status	Final
Previous versions	Unaudited draft January 2003
Number of pages	97
Number of tables	9
Number of figures	2
Number of annexes	6
Number of appendices	7

Summary

The inhalation toxicity of aerosols of **NOCOLOK™ flux** was studied in a sub-acute (28-day) study in male Wistar rats. Groups of 6 male rats were exposed to target concentrations of 0 (control), 1, 3, 10, or 100 mg/m^3 **NOCOLOK™ flux** for six hours a day, 5 days a week during a period of 4 weeks, with a total of 20 exposure days. The rats were necropsied the day after the last exposure. To examine the toxicity of the test material clinical signs, body weights, food consumption, food conversion efficiency, haematology, and clinical chemistry were used. In addition, a full necropsy was performed and a selection of organs including the respiratory tract, was examined microscopically.
The study was expanded with a sensitisation study to examine sensitising properties of **NOCOLOK™ flux** in Brown Norway rats. The results of this study are reported in a separate report (V4671/02).

The mean actual concentrations (± standard deviation) of **NOCOLOK™ flux** in the test atmospheres were 1.00 (0.13), 3.10 (0.24), 10.3 (1.2), and 103.8 (6.6) mg/m^3, for the low, lower mid, higher mid and high concentration, respectively. The (mean) MMAD (Mass Median Aerodynamic Diameter) of the particles in the aerosols were 1.5, 2.0, 1.4, and 2.5 μm for the low, lower mid, higher mid and high concentration, respectively, with mean geometric standard deviations of 2.4, 2.0, 2.1, and 1.7, respectively.

No treatment-related abnormalities were observed.

No treatment-related changes in body weight gain were noted.

Food consumption and food conversion efficiency in exposure groups tended to be lower than in controls.

No treatment-related changes in haematology and clinical chemistry were observed.

Concentration-related statistically significant increases in absolute and relative lung weight were observed in rats exposed to 3, 10 or 100 mg/m^3.

Macroscopic examination at necropsy did not reveal treatment-related changes.

Inhalation of **NOCOLOK™ flux** induced histopathological changes in the nasal passages, larynx, and lungs:
- Focal olfactory epithelial necrosis was observed in all animals exposed to 100 mg/m^3, in one animal exposed to 10 mg/m^3, and in one animal exposed to 3 mg/m^3. Focal vacuolation of the olfactory epithelium, possibly a precursor of necrosis was observed in a few animals exposed to 10 mg/m^3, and in one animal exposed to 3 mg/m^3. Respiratory epithelial metaplasia was observed in all animals exposed to

100 mg/m^3, goblet cell hyperplasia of the respiratory epithelium was observed in a few animals exposed to 10 mg/m^3. No treatment-related lesions were observed in the anterior part of the nose.
- Sqaumous metaplasia of the larynx with an underlying granulomatous inflammation was seen in animals exposed to 100 mg/m^3.
- In the lungs, treatment-associated lesions consisted of typical alveolar macrophage accumulations, accompanied by cellular debris/material lying freely in the alveolar lumen (all test groups), inflammation (3, 10 and 100 mg/m^3 test groups) and bronchial/bronchiolar epithelium alterations (3, 10 and 100 mg/m^3 test groups). In addition, the incidence of BALT germinal centre development increased with the concentration.

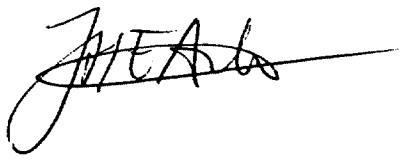
Conclusion

From the results of the present study in rats, it was concluded that exposure to **NOCOLOK™ flux** at levels of 3 mg/m^3 and higher induced increased absolute and relative lung weights, and histopathological changes in the nose and in the lungs, including typical alveolar macrophage accumulations. Typical alveolar macrophages, however, were also observed in animals exposed to 1 mg/m^3. The additional presence of cellular debris/material in the alveolar lumina of a few of these animals suggests impaired or insufficient clearance capacity of the alveolar macrophages, which is considered to be an adverse reaction to the exposure with the test compound. A No-Observed-Effect-Level (NOEL) could, therefore, not be established. However, as the number of accumulated macrophages was small and there was only a tiny amount of deposited material/cellular debris, the concentration of 1 mg/m^3 was considered to be a Minimal-Observed-Adverse-Effect Level (MOAEL).

Contents

Statement of GLP Compliance

We, the undersigned, hereby declare that this report constitutes a true and complete representation of the procedures followed and of the results obtained in this study by TNO Nutrition and Food Research Institute, and that the study was carried out under our supervision.
The study was carried out in accordance with the OECD Principles of Good Laboratory Practice.



Dr Ir J.H.E. Arts
(Study director)

22 May 2003
Date

Drs H.H. Emmen
(Management, Head Department of
Target Organ Toxicology)

22 May, 2003
Date

Authentication of co-operating scientists

I, the undersigned, hereby declare that the pathology data presented in this report were compiled by me or under my supervision, and accurately reflect the data obtained.

Dr R.A. Woutersen

Dr C.F. Kuper
(Pathologist)

Head, Dept of General Toxicology

22 May 2003
Date:

Quality Assurance Statement

On: A sub-acute (28_day) inhalation toxicity study with NOCOLOK™ flux in male Wistar rats

Report Number: V4671/01

Date : May 2003

The protocol of this study was inspected by the Quality Assurance Unit of TNO Nutrition and Food Research Institute as follows:

Date of inspection:
8 July 2002

Date of report:
8 July 2002

The experimental phase was inspected as follows:

Date of inspection:
8 July 2002

Date of report:
8 July 2002

This report was audited as follows:

Date of audit:
17 March 2003 (draft report)
22 May 2003 (final report)

Date of report:
31 March 2003
22 May 2003

I, the undersigned, hereby declare that this report provides an accurate record of the procedures employed and the results obtained in this study; all inspections were reported to the study director and the management on the dates indicated.

Ing P.A. de Lang
(Quality Assurance Auditor)

Date: 22 May 2003

GLP compliance monitoring unit statement

inspectie W&V

ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF
GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 88/320/EEC the conformity with the OECD Principles of GLP was assessed on 22-26 November1999 at

TNO Nutrition and Food Research Institute
Utrechtseweg 48
P.O. Box 360
3700 AJ Zeist

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following areas of expertise: Toxicity and Mutagenicity studies, and studies on Metabolism and Kinetics.

The Hague, 23 December 1999

RLA

Th. Helder, DVM
GLP Compliance Monitoring Unit

Testing facility

The toxicity study was conducted by:

TNO Nutrition and Food Research
P.O. Box 360, 3700 AJ ZEIST, the Netherlands
Telephone +31 30 69 44 144
Telefax +31 30 69 60 264
Visitors address: Utrechtseweg 48, Zeist, the Netherlands

Contributors

Major contributions to this study were made by:

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Study Assistant:	A. van Garderen-Hoetmer[2]
Deputy Study Director:	Dr H. Muijser[1]
Inhalation techniques:	Ing E. Duistermaat[1]
Biotechniques:	G. van Beek[2]
Haematology and clinical chemistry:	J.F. Catsburg[2]
Histotechniques:	E.C.M. van Oostrum[2]
Pathologist:	Dr C.F. Kuper[2]

[1] Department of Target Organ Toxicology, TNO Nutrition and Food Research Institute
[2] Department of General Toxicology

1 Introduction

At the request of Solvay SA, Bruxelles, Belgium, a sub-acute (28-day) inhalation toxicity study with **NOCOLOK™ flux** was carried out in Wistar rats, to obtain data on the sub-acute toxicity of this compound upon repeated inhalatory exposure and to establish a no-observed-adverse-effect level (NOAEL). This study is a follow-up study of an earlier sub-acute inhalation toxicity study with NOCOLOK™ flux at concentrations of 100, 300 and 600 mg/m^3. Since a No-Observed-Adverse-Effect Level (NOAEL) could not be found in that study (TNO report V99.283), the purpose of the present study was to examine toxicity at lower concentration levels. Since no sex differences were observed in the first study, it was not considered necessary to study both sexes, and male rats were chosen for the present study.
In addition, the study was expanded with a sensitisation study to examine sensitising properties of NOCOLOK™ flux in Brown Norway rats. The results of this study are reported separately (TNO Report V4671/02).

2 Experimental

The study was conducted according to a protocol, entitled: "Protocol for a sub-acute (28-day) inhalation toxicity study with NOCOLOK™ flux, including a sensitisation study, in rats", approved by the study director on 28 June 2002. The protocol had been drafted in accordance with the following guidelines:
- OECD Guideline for Testing of Chemicals no. 412, adopted 12 May 1981
- EC guideline no. B8, EEC Directive 92/69/EEC, Official Journal of the European Communities, no. L383 A, 29.12.92.

2.1 Test material

The test material was supplied by the sponsor. Two white plastic containers with the test material, labelled 'Nocolok(R)Flux, Kaliumaluminiumfluorid Fein', gross weights 5487.50 and 5485.66 g, respectively, were received in good condition on 3 June 2002. The TNO internal reference number was 020099. The test material was stored at room temperature.

NOCOLOK™ flux is a white powder, and has the following characteristics (as given by the sponsor):

Name of test material:	**NOCOLOK™ flux**
Chemical name:	$AlKF_4$
Batch number:	AB010701
Purity:	>99%
Expiry date:	1 January 2003
Storage conditions:	room temperature

For technical reasons **NOCOLOK™ flux** will be abbreviated in the remaining part of the report as **NOCOLOK flux**.

2.2 Test system

2.2.1 Characterization of the test system

The study was conducted with rats. This species was used because it is considered most suitable for this type of study and is usually required by regulatory agencies. Young, male Wistar derived rats (Crl:(WI)WU BR) were obtained from a colony maintained under SPF-conditions at Charles River Deutschland, Sulzfeld, Germany. The animals, 33 males, arrived on 26 June 2002 when they were about 6 weeks old. Upon arrival they were taken in their unopened shipping boxes to their definitive animal room (room number 6.0.08), checked for overt signs of ill health and anomalies. They were kept in quarantine upon approval of the lot (negative titres to microorganisms tested) by checking their microbiological status by the conduct of serological controls in a few randomly chosen animals. The first exposure started on 3 July 2002, therefore the animals were acclimatized to the laboratory conditions in the animal room for 7 days.

2.2.2 Animal allocation and identification

On 28 June 2002, the rats were identified with a temporary tail mark and weighed. They were weighed again on 2 July 2002, one day before the start of exposure, checked for adequate growth and weight variation (< 20 % of the mean weight) and were allocated to five groups of 6 rats, proportionately by weight class, by a computer randomization programme. Subsequently, each rat was uniquely identified by an animal number tattooed in the ears (see Annex 1). Finally 30 male animals were placed in their definitive cages, 3 males per cage. The remaining 3 male animals were kept as sentinel animals.

2.3 Experimental conditions

2.3.1 Animal maintenance

Housing conditions were conventional. Mean temperature and relative humidity in the animal room, monitored continuously, were 20.8 °C and 61%, respectively. The temperature was between 19.8 and 24.3°C, and the relative humidity between 52 and 98%. High relative humidity values were observed during cleaning periods. High relative humidity values were also observed on a few days for longer periods, i.e during ca. 1 hour on 24 July, ca. 4 hours on 30 July and during ca. 13 hours on 31 July 2002.
The number of air changes was about 10 per hour. Lighting was artificial by fluorescent tubes, time switch controlled at a sequence of 12 hours light and 12 hour dark.

The living cages were allocated to the various groups (Annex 1). Each cage was provided with a coloured card showing the animal identification number range, the group letter and the study number.

During exposure the animals had no access to feed or water and were housed individually in restraining tubes which, in turn, were placed in nose-only exposure units (see section 2.5). The tubes were identified by the animal identification number, the group letter and the colour code. Immediately after exposure, the animals were returned to their living cages.

2.3.2 Feed and drinking water

Feed and drinking water were provided *ad libitum* from the arrival of the rats until the end of the study (except during exposure). The feed was provided as a powder in stainless steel cans, covered by a perforated stainless steel plate which prevented spillage. The animals were fed a commercially available rodent diet (Rat & Mouse No. 3 Breeding Diet RM3) from SDS Special Diets Services, Witham, England. Each batch of this diet is analysed by SDS for nutrients and contaminants. A copy of the certificates of analysis pertaining to the batch used (Batch no. 2206) is attached to this report as Annex 2.
Drinking water was given in bottles, which were cleaned weekly and filled up when necessary. Tap water for human consumption (quality guidelines according to Dutch legislation based on EEC Council Directive 98/83/EEC) was supplied by N.V. Hydron Midden-Nederland. Results of the routine physical, chemical and microbiological examination of drinking water as conducted by the supplier are made available to TNO Nutrition and Food Research Institute. In addition, the supplier periodically (twice per year) analyses water samples taken on the premises of TNO in Zeist for a limited number of physical, chemical and microbiological variables. The results of the samples taken during or close to the conduct of this study are presented in Annex 3.

2.4 Experimental procedures

2.4.1 Frequency and duration of exposure to the test material

The rats were exposed to the test material for 6 hours a day, 5 days a week for a period of 28 days, resulting in a total number of 20 exposure days.
The study was started on 3 July 2002 with the first exposure of the animals and was finished with the necropsy of the rats on 31 July 2002.

2.4.2 Exposure levels

Based on the results of an earlier sub-acute inhalation toxicity study with NOCOLOK™ flux at concentration levels of 100, 300 and 600 mg/m^3 (TNO report V99.283), in consultation with the sponsor, the following levels were chosen: 0, 1, 10, and 100 mg/m^3.

2.4.3 Number and size of test groups

The study was identified with a computer study number (4671/01). The study comprised five groups, one control and four concentration groups, each consisting of 6 male rats. The table below shows group code, colour code, exposure level and number of animals in each group.

Group	Colour code	Concentration in air (mg/m^3)	Number of rats (males/females)
A (control)	white	0	6
B (low-concentration)	blue	1	6
C (mid-concentration I)	green	3	6
D (mid-concentration II)	red	10	6
E (high-concentration)	yellow	100	6

2.5 Exposure units

Animals were exposed to the test atmosphere in nose-only inhalation units, a modification of the chamber manufactured by ADG Developments Ltd., Codicote, Hitchin, Herts. SG4 8UB, United Kingdom (see Figure 1). Each unit consisted of a cylindrical column, surrounded by a transparent cylinder. The column had a volume of ca. 50 ℓ and consisted of a top assembly with the inlet of the test atmosphere, a rodent tube section and at the bottom the base assembly with the exhaust port. The rodent tube section had 20 ports for animal exposure. Several empty ports were used for test atmosphere sampling, particle size analysis,
temperature and relative humidity. The animals were secured in plastic animal holders (Battelle), positioned radially through the outer cylinder around the central column. The remaining ports were closed. Only the nose of the rats protruded into the interior of the column.
In our experience, the animal's body does not exactly fit in the animal holder which always results in some leak from high to low pressure side. By securing a positive pressure in the central column and a slightly negative pressure in the outer cylinder, which encloses the entire animal holder, air leaks from nose to thorax rather than from thorax to nose and dilution of test atmosphere at the nose of the animals is prevented. Control rats were also placed in Battelle restraining tubes and exposed to humidified pressurized air in a similar nose-only inhalation unit. The units were illuminated externally by normal laboratory TL-lighting.

2.6 Generation of the test atmosphere

The inhalation equipment was designed to expose the animals to a continuous supply of fresh test atmosphere. The high concentration test atmosphere was

generated by passing the test material to an eductor (Fox Mini, type 060, Spraybest Europe BV, Zwanenburg, The Netherlands) using a dry material feeder (Gericke GMD 60, Gericke AG, Regensburg-Zürich, Switzerland). The test material was aerosolized in the eductor, which was supplied by humidified pressurized air and was placed at the top inlet of the high concentration exposure unit (unit E). From there the test atmosphere was directed towards the animal noses. Also, however, parts of the test atmosphere were extracted by using eductors (Fox Mini, type 060 for unit B, and Fox Mini, type 031 for units C and D; Spraybest Europe BV, Zwanenburg, The Netherlands) to transport and to dilute the test atmosphere for the low, lower mid and higher mid concentration test atmospheres, respectively. The resulting test atmospheres of units C and D were further diluted by flows of unhumidified air using mass flow controllers (Bronkhorst, HiTec, Ruurlo, The Netherlands) starting from the 2nd exposure day (4 July 2002) for unit C and from the 11th exposure day (17 July 2002) for unit D. At the outlet of the units, the test atmosphere was exhausted (see also Figure 1). Before the first exposure and after the last exposure, the air flow through the eductors were measured for a range of input pressures.

2.7 Analysis of the test atmosphere

2.7.1 Actual concentration

The concentration of the test material in each test atmosphere was determined by gravimetric analysis. For the high and higher mid concentration test atmosphere, these measurements were carried out three times each exposure day. For the lower mid and low concentration, these measurements were carried out two times and once per exposure day, respectively. The sample for the low concentration test atmosphere took almost the whole exposure period. On one occasion, however, the number of determinations/day in the high concentration test atmosphere was four (see Table 1.1).
Representative samples from the test atmospheres were obtained by passing ca. 50 ℓ (high concentration), ca. 150-300 ℓ (higher mid concentration), ca. 600 ℓ (lower mid concentration), or ca. 1500 ℓ (low concentration) test atmosphere samples at 5 ℓ/min through fibre glass filters (Sartorius, 13430-44-S). Filters were weighed before sampling, loaded with aerosol particles and weighed again.

As only one gravimetry sample of the low concentration test atmosphere could be taken per day, the stability of the test atmosphere was monitored using a condensation nucleus counter (CNC, type 3020, TSI Inc., St Paul, MN, USA).

2.7.2 Air flow

The input pressures of the eductors and the settings of the mass flow controllers (exposure units) and the reading of the rotameter (control unit) were recorded at the start of each exposure day. The input pressures of the eductors and the settings of the mass flow controllers were also recorded at the end of each exposure day.

If necessary and applicable, based on results of gravimetric analyses, pressures were adapted accordingly to achieve the target concentrations. As no changes were made in the setting of the rotameter of the control unit, this was recorded only once per day. In this way, total air flow during exposure was monitored, in part indirectly, through the aerosol generation system.

2.7.3 Nominal concentration

The nominal concentration in the high concentration test atmosphere was computed by dividing the amount of test material used (by weight) on a weekly basis by the total volume of air passed through the inhalation unit during that period. As the amounts taken from the high concentration test atmosphere to generate the lower concentration test atmospheres could not be estimated, nominal concentrations for these lower concentrations could not be calculated.

2.7.4 Particle size measurement

Measurements of the particle size distribution were carried out using a 10-stage cascade impactor (Andersen, Atlanta, USA) with a largest cut-off size of 32 μm. Particle size distribution measurement was carried out three times during the study period on the 10 and 100 mg/m^3 test atmospheres, and two times on the 3 mg/m^3 test atmosphere. Because the sampling period took more than a one-day exposure period of the 1 mg/m^3 test atmosphere, particle size distribution measurement was carried out once during the experimental period during three consecutive exposure days to compare the results with those obtained for the 3 and 10 mg/m^3 atmosphere. The Mass Median Aerodynamic Diameter (MMAD) and the geometric standard deviation (gsd) were calculated (Lee, 1972).

2.7.5 Measurement of temperature and relative humidity

The temperature and the relative humidity of the test atmosphere were recorded about once every hour (5-6 times/day) during exposure using a RH/T device (TESTO 610, GmbH & Co, Lenzkirch, Schwarzwald, Germany).

2.8 Observations and measurements

2.8.1 Clinical signs

Each animal was observed daily in the morning hours by cage-side observations and, if necessary, handled to detect signs of toxicity. All animals were checked again in the afternoon (shortly after exposure) especially for dead or moribund animals, to minimise loss of animals from the study. At weekend days only one check per day was carried out. All abnormalities, signs of ill health or reactions to treatment were recorded.

2.8.2 Body weights

The body weight of each animal was recorded five days before the start of the first exposure (day -5), one day before the start of the first exposure (day -1; allocation procedure), just prior to the first exposure (day 0), on days 7, 14, 21, and on their scheduled sacrifice date (day 28) in order to calculate the correct organ to body weight ratios.

2.8.3 Food consumption and food conversion efficiency

Food consumption of the animals was measured per cage by weighing the feeders. The consumption was measured over three successive periods of 7 days, and one 6-day period, starting on day 0. The results are expressed in g per animal per day. The efficiency of food utilization was calculated and expressed in g weight gain per g food consumed.

2.8.4 Haematology

Haematology was conducted at the end of the exposure period (day 28). At scheduled necropsy, blood samples were taken from the abdominal aorta of the (overnight fasted) rats whilst under ether anaesthesia. About the first ml of blood was collected using K_2-EDTA as anticoagulant. In each sample the following determinations were carried out according to the methods listed in Annex 4:

- haemoglobin
- packed cell volume
- red blood cell count
- reticulocytes
- total white blood cell count
- differential white blood cell count
- prothrombin time
- thrombocyte count

The following parameters were calculated:
- mean corpuscular volume (MCV)
- mean corpuscular haemoglobin (MCH)
- mean corpuscular haemoglobin concentration (MCHC).

2.8.5 Clinical chemistry

At scheduled necropsy, the day after the last exposure (day 28), blood was collected from the abdominal aorta of all, overnight fasted, animals whilst under nembutal anaesthesia. The blood was collected in heparinized plastic tubes and plasma was prepared by centrifugation. The following measurements were made in the plasma according to the methods listed in Annex 5:

- alkaline phosphatase activity (ALP)
- aspartate aminotransferase activity (ASAT)
- alanine aminotransferase activity (ALAT)
- gamma glutamyl transferase activity (GGT)
- total protein
- albumin
- ratio albumin to globulin
- urea
- creatinine
- fasting glucose.
- bilirubin total
- cholesterol
- triglycerides
- phospholipids
- calcium (Ca)
- sodium (Na)
- potassium (K)
- chloride (Cl)
- inorganic phosphate

2.8.6 Pathology

Gross necropsy and tissue collection
At the end of the exposure period (day 28), the animals were killed by exsanguination from the abdominal aorta under nembutal anaesthesia and then examined grossly for pathological changes, including examination of the teeth. The sequence used was balanced for groups.

The underlined organs were weighed (paired organs together; see below) as soon as possible after dissection to avoid drying. The relative organ weights (g/kg body weight) were calculated based on the final body weight of the rats.

Samples of the following tissues and organs of all animals were preserved in a neutral aqueous phosphate-buffered 4 per cent solution of formaldehyde (10% solution of formalin). The lungs (after weighing) were infused with the fixative under ca. 15 cm water pressure to insure fixation.

<u>adrenals</u>
<u>brain</u>
<u>heart</u>
<u>kidneys</u>
<u>liver</u>
<u>spleen</u>
<u>testes</u>
<u>lungs with trachea and larynx</u>
nasal passages (including upper teeth)
all gross lesions

Histopathological examination
The tissues required for microscopic examination were embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin.
The respiratory tract (nose, larynx, trachea and lungs) was processed as follows: The nose (nasal cavity) was cut at 6 levels. Levels of cross sections through the nasal cavity were assigned according to international standards (Woutersen et al., 1994: Young, 1986; Annex 6) The larynx was cut longitudinally. The trachea with the bifurcation was cut longitudinally alongside the bifurcation. Each lung lobe was sectioned.

Histopathological examination was performed on the respiratory tract of all animals of all groups since treatment-related changes were observed in animals of the high

concentration group. In addition, the gross lesions observed in the control group (group A) and the high concentration group (group E) were examined microscopically.

2.9 Statistical analysis

Body weight data were analysed by one-way analysis of covariance (ANCOVA) using pre-exposure (day 0) weights as the covariate. Red blood cell and coagulation variables, total white blood cell counts, absolute differential white blood cell counts, clinical chemistry values, and organ weights were analysed by one-way analysis of variance (ANOVA). When group means were significantly different ($p<0.05$), individual pairwise comparisons were made using Dunnett's multiple comparison method (Cochran, 1957; Steel and Torrie, 1960; Dunnett, 1955 and 1964). Relative differential white blood cell counts were analysed by Kruskal-Wallis non-parametric Anova followed by Mann-Whitney U-test. The incidences of histopathological changes were evaluated by Fisher's exact probability test (Siegel, 1956).
All pairwise comparisons were two tailed. Group mean differences with an associated probability of less than 0.05 were considered to be statistically significant. Because numerous variables were subjected to statistical analysis, the overall false positive rate (Type I errors) may be greater than suggested by a probability level of 0.05. Therefore, the final interpretation of results was based not only on statistical analysis but also on other considerations such as dose-response relationships and whether the results were significant in the light of other biological and pathological findings.

2.10 Retention of records, samples and specimens

A reference sample of the test material, raw data, the master copy of the final report and all other information relevant to the quality and integrity of the study, including tissue specimens, paraffin and epoxy resin embedded blocks and microscopic slides, were stored in the archives of the TNO Nutrition and Food Research Institute and will be retained for a period of at least five years (tissue specimens, paraffin and epoxy resin embedded blocks), 10 years (reference sample of the test substance) or at least 15 years (slides, raw data) after reporting of the study. At the end of the five year storage period, the sponsor will be asked whether the tissue specimens and paraffin and epoxy resin embedded blocks can be discarded, should be stored for an additional period, or transferred to the archives of the sponsor.

2.11 Deviations from the protocol

- In the protocol concentrations were accidentally mentioned in g/m^3 rather than mg/m^3.
- Besides during cleaning periods, high relative humidity values (above 70%) were also observed on a few days for longer periods, i.e during ca. 1 hour on 24 July, ca. 4 hours on 30 July and during ca. 13 hours on 31 July 2002.
- Particle size distribution measurements in the high and higher mid concentration

test atmospheres were carried out three times during the study period instead of every week. Particle size distribution measurements in the lower mid concentration test atmosphere were carried out two times during the study period instead of once. Particle size distribution measurement in the low concentration test atmosphere was also carried out but this needed a three-day sampling period.

- As the amounts taken from the high concentration test atmosphere to generate the lower concentration test atmospheres could not be estimated, nominal concentrations for these lower concentrations could not be calculated.
- The air flow through the units was recorded by the setting of the rotameter (control unit) and pressures of eductors and bypass flows (exposure units) at the start and the end of each exposure day. Therefore recordings were made twice each day instead of three times per day.
- The temperature and relative humidity were generally measured three times per day instead of every hour.

The deviations above are not considered to have influenced the validity of the study.

3 Results

3.1 Analytical results

3.1.1 Actual concentration (Table 1.1)

The overall mean daily concentrations and their standard deviations were 1.00 ± 0.13 mg/m³, 3.10 ± 0.24 mg/m³, 10.3 ± 1.2 mg/m³, and 103.8 ± 6.6 mg/m³, for the low, lower mid, higher mid, and high concentration test atmospheres, respectively (Table 1.1). These overall mean concentrations were very close to the target concentrations of 1, 3, 10 and 100 mg/m³.

As only one gravimetry sample of the low concentration test atmosphere could be taken per exposure day, the stability of the test atmosphere was monitored using a condensation nucleus counter. A stable test atmosphere was obtained.

3.1.2 Airflow (Table 1.2)

Air flows (Table 1.2) were 32.7, 53.1, 52.9, 32.1, and 128.1 ℓ/min for the control, low, lower mid, higher mid, and high concentration test atmospheres, respectively.

3.1.3 Nominal concentration (Table 1.3)

Nominal concentrations of the high concentration test atmosphere and its mean are listed in Table 1.3. Due to the exposure set up, nominal concentration was measured on a weekly basis. The overall mean value was 127 mg/m³, indicating a generation efficiency of 81%, which is considered high for a dust type test atmosphere.

3.1.4 Particle size distribution (Table 1.4)

The particle size distributions of the test atmospheres are indicated in Table 1.4. For the high concentration test atmosphere the MMAD was 2.5 μm (measured 3 times), with a geometric standard deviation (gsd) between 1.6 and 1.8 (mean 1.7). For the higher mid concentration test atmosphere the MMAD was between 1.3 and 1.6 μm (measured 3 times; mean 1.4 μm), and the gsd between 1.9 and 2.2 (mean 2.1). For the lower mid concentration test atmosphere the MMAD was 1.8 and 2.2 μm (measured 2 times; mean 2.0 μm), and the gsd was 1.9 and 2.0 (mean 2.0). For the low concentration test atmosphere the MMAD was measured only once during three consecutive exposure days. The MMAD was 1.5 μm and the gsd 2.4.

3.1.5 Temperature and relative humidity (Appendices 1.1 and 1.2)

The mean daily temperatures in the test atmospheres (see Appendix 1.1) were 21.9 ± 0.3°C, 22.1 ± 0.3°C, 22.3 ± 0.3°C, 22.5 ± 0.4°C, and 22.7 ± 0.3°C for the control, low, lower mid, higher mid, and high concentration test atmosphere, respectively.

The mean daily relative humidity values (see Appendix 2.2) were 32.4 ± 0.9%, 32.6 ± 0.6%, 34.4 ± 1.4%, 36.0 ± 1.5%, and 41.0 ± 2.0% for the control, low, lower mid, higher mid, and high concentration test atmospheres, respectively.

3.2 Clinical signs and survival (Table 2; Appendix 2)

Individual observations in the mornings, i.e. before the start of each day's exposure did not reveal treatment-related changes. There was one male exposed to 10 mg/m^3 that showed malocclusion of incisors from day 3 until day 6 of the study (Table 2 and Appendix 2).

All rats survived until their scheduled necropsy.

3.3 Body weights (Table 3; Appendix 3)

Statistically significant changes in body weight gain were not observed (Table 3).

3.4 Food consumption and food conversion efficiency (Tables 4.1 and 4.2)

Within the exposure groups, food consumption and food conversion efficiency tended to be lower than that in controls (Tables 4.1 and 4.2).

3.5 Haematology (Tables 5.1-5.3; Appendices 4.1-4.3)

No changes were observed in red blood cell parameters (Table 5.1). Determination of white blood cell parameters showed a slight but statistically significant increase in the absolute number of basophils in animals of the low concentration group only. This was considered to be an isolated finding as no such change was observed in animals exposed to higher concentrations (Table 5.2).

3.6 Clinical chemistry (Tables 6.1-6.3; Appendices 5.1-5.3)

No changes in clinical chemistry parameters were observed (Table 6).

3.7 Organ weights (Tables 7.1 and 7.2; Appendices 6.1 and 6.2)

A concentration-related increase in absolute and relative lung weight was observed in male rats of all test groups. With respect to both absolute and relative lung weight, statistical significance was reached in animals exposed to 3, 10, or 100 mg/m^3 (Table 7). In the other organs, significant weight differences between control and exposed animals were not detected.

3.8 Pathology (Tables 8 and 9; Appendix 7)

3.8.1 Macroscopic examination (Table 8; Appendix 7)

Macroscopic examination at necropsy did not reveal treatment-related changes.

3.8.2 Microscopic examination (Table 9; Appendix 7)

Inhalation of NOCOLOK flux induced histopathological changes in the nasal passages, larynx and lungs.

The nasal lesions occurred predominantly in the olfactory epithelium (OE). The OE of all animals exposed to 100 mg/m^3, of one animal exposed to 10 mg/m^3, and of one animal exposed to 3 mg/m^3 had disappeared completely focally on the septum at levels 5 and 6 and at various sites of the ecto- and endoturbinates (reported as 'focal olfactory epithelial necrosis'). The submucosa at the sites of OE necrosis was oedematous, with gland-like epithelial structures, loss of nerves and a mixed inflammatory cell infiltration, whereas the epithelium bordering the necrosis was thinned and flattened. In a few animals exposed to 10 mg/m^3 and in one animal exposed to 3 mg/m^3, focal vacuolation of OE was observed. This change may be a precursor lesion of necrosis. Furthermore, metaplasia of OE to respiratory-like epithelium or expansion of respiratory epithelium (RE) at the expense of OE (denoted collectively as 'respiratory epithelial metaplasia') was observed at levels 5 and 6 in all animals exposed to 100 mg/m^3.
All animals exposed to 100 mg/m^3 and a few animals exposed to 10 mg/m^3 exhibited goblet cell hyperplasia of RE in the ventral meatus.
No treatment–related lesions were observed in the anterior part of the nasal passages.

The treatment-related histopathological lesions in the larynx were observed predominantly at the base of the epiglottis of animals exposed to 100 mg/m^3. However, this small part of the larynx was not always included suffciently in the section to examine the lesions properly (denoted as 'area of concern not included in the section', see Appendix 7). All animals exposed to 100 mg/m^3 with an adequately sectioned larynx (3 out of 6 animals) showed metaplasia of respiratory epithelium to squamous epithelium, and an underlying granulomatous inflammation with cell necrosis and mineralisation. Several animals in the other groups, including controls, also exhibited squamous metaplasia. However, this was observed at the mid portion of the epiglottis as patches of squamous epithelium intermixed with respiratory epithelium in the mid portion of the epiglottis. This was considered to be a normal finding, unrelated to exposure.
In addition, one animal exposed to 100 mg/m^3 exhibited a microcyst with, partly birefringent, particulate material, that may be test material, in the laryngeal epithelium, at the level of the cricoid cartilage.

The treatment-associated lung lesions consisted of typical alveolar macrophage accumulations (all test groups), inflammation (3, 10 and 100 mg/m^3 test groups) and

bronchial/bronchiolar epithelium alterations (3, 10 and 100 mg/m^3 test groups). The alveolar macrophages observed in all test groups differed in morphology and numbers from the alveolar macrophages seen in one of the controls. The macrophages found in the exposed animals were large with pale foamy to slightly eosinophilic cytoplasm, occasionally containing basophilic inclusions/granules in animals exposed to 3 mg/m^3. These were accompanied by a few granulocytes in animals exposed to 3, 10 or 100 mg/m^3. Pale foamy material, suggestive of macrophage disintegration, laid freely in the lumen of alveoli of animals of all test groups. This was also observed in a few animals exposed to 1 mg/m^3, although the amount of this material was very slight.
In some areas of the lungs, particularly in animals exposed to 100 mg/m^3, the increased eosinophilia and the amount of free alveolar material was strongly suggestive for alveolar (lipo)proteinosis, a condition in which impaired activity of the alveolar macrophages and overproduction of surfactant is suspected. The number of alveoli involved and the number and size of alveolar macrophages increased with concentration of the test compound, although in some animals exposed to 100 mg/m^3, the size of the macrophages appeared to be decreased when compared to animals exposed to 3 or 10 mg/m^3.
Treatment-related inflammation varied from focal alveolitis (interstitial inflammatory cell infiltrate with increased numbers of alveolar macrophages and granulocytes) in animals exposed to 3 or 10 mg/m^3 to more diffuse alveolitis in animals exposed to 100 mg/m^3. Moreover, the incidence and degree of cuffs of mononuclear inflammatory cells around bloodvessels ('perivascular mononuclear cell infiltrate') increased with the concentration from the group of animals exposed to 3 mg/m^3 or higher. In addition, the incidence of BALT germinal centre development increased with the concentration. Bronchial/bronchiolar hypertrophy was observed in several animals exposed to 3 mg/m^3 and in a single animal exposed to 10 or 100 mg/m^3.

Microscopic examination of the grossly enlarged parathymic lymph nodes in the thoracic cavity of one 100 mg/m^3 animal demonstrated aggregates of large macrophages with basiophilic inclusions/granules.

4 Discussion and conclusion

The results of the present subacute (28-day) inhalation toxicity study with **NOCOLOK flux** indicated that exposure of male rats for 6 h/day, 5 d/week for 28 days at concentrations of 1, 3, 10 or 100 mg/m^3, induced changes in the respiratory tract. As the particle size distribution measurements showed particle sizes with (mean) MMADs of 1.5, 2.0, 1.4, and 2.5 μm for the low, lower mid, higher mid, and high concentration test atmosphere, respectively, significant amounts of test material had reached the lungs as shown by the observed lung effects.

Nasal effects consisted of focal olfactory epithelial necrosis in all animals exposed to 100 mg/m^3, in one animal exposed to 10 mg/m^3, and in one animal exposed to 3 mg/m^3. Focal vacuolation of the olfactory epithelium, possibly a precursor of necrosis, was observed in a few animals exposed to 10 mg/m^3, and in one animal exposed to 3 mg/m^3. Further, respiratory epithelial metaplasia was observed in all animals exposed to 100 mg/m^3, and goblet cell hyperplasia of the respiratory epithelium was observed in a few animals exposed to 10 mg/m^3. In the larynx, changes were limited to animals exposed to 100 mg/m^3, showing squamous metaplasia with an underlying granulomatous inflammation. In the lungs, treatment-associated lesions consisting of typical alveolar macrophage accumulations were seen in all test groups. Inflammation and bronchial/ bronchiolar epithelium alterations were observed in animals exposed to 3, 10 or 100 mg/m^3. In addition, the incidence of BALT germinal centre development increased with the concentration.
In itself, alveolar macrophage accumulation is not an adverse effect. The alveolar macrophages, however, differed in morphology and numbers from the alveolar macrophages seen in one of the controls. The macrophages found in the exposed animals were large with pale foamy to slightly eosinophilic cytoplasm. Pale foamy material, suggestive of macrophage disintegration, laid freely in the lumen of alveoli of animals of all test groups. In a few animals exposed to 1 mg/m^3, the numbers of accumulated macrophages were only slightly increased and the amount of deposited material was minimal.

The adverse effects found in the respiratory tract seemed to be consistent with the increases in absolute and relative lung weight found in animals exposed to 3 mg/m^3 or higher.

Body weight and food consumption were unchanged and exposure related clinical signs in the mornings, before each exposure session were not seen.

From the results of the present study in rats, it was concluded that exposure to **NOCOLOK™ flux** at levels of 3 mg/m^3 and higher induced increased absolute and relative lung weights, and histopathological changes especially in the nose and lungs, including typical alveolar macrophage accumulations. The latter, however, were also observed in animals exposed to the lowest concentration of 1 mg/m^3.
The additional presence of cellular debris/material lying freely in the alveolar lumina

of a few of these animals suggests impaired or insufficient clearance capacity of the alveolar macrophages, which is considered to be an adverse reaction to the exposure with the test compound. A No-Observed-Effect-Level (NOEL) could, therefore, not be established. However, as the number of accumulated macrophages was small and there was only a tiny amount of deposited material/cellular debris, the concentration of 1 mg/m^3 was considered to be a Minimal-Observed-Adverse-Effect Level (MOAEL).

5 References

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Figures

Figure 1 - Schematic diagram of the generation and exposure system

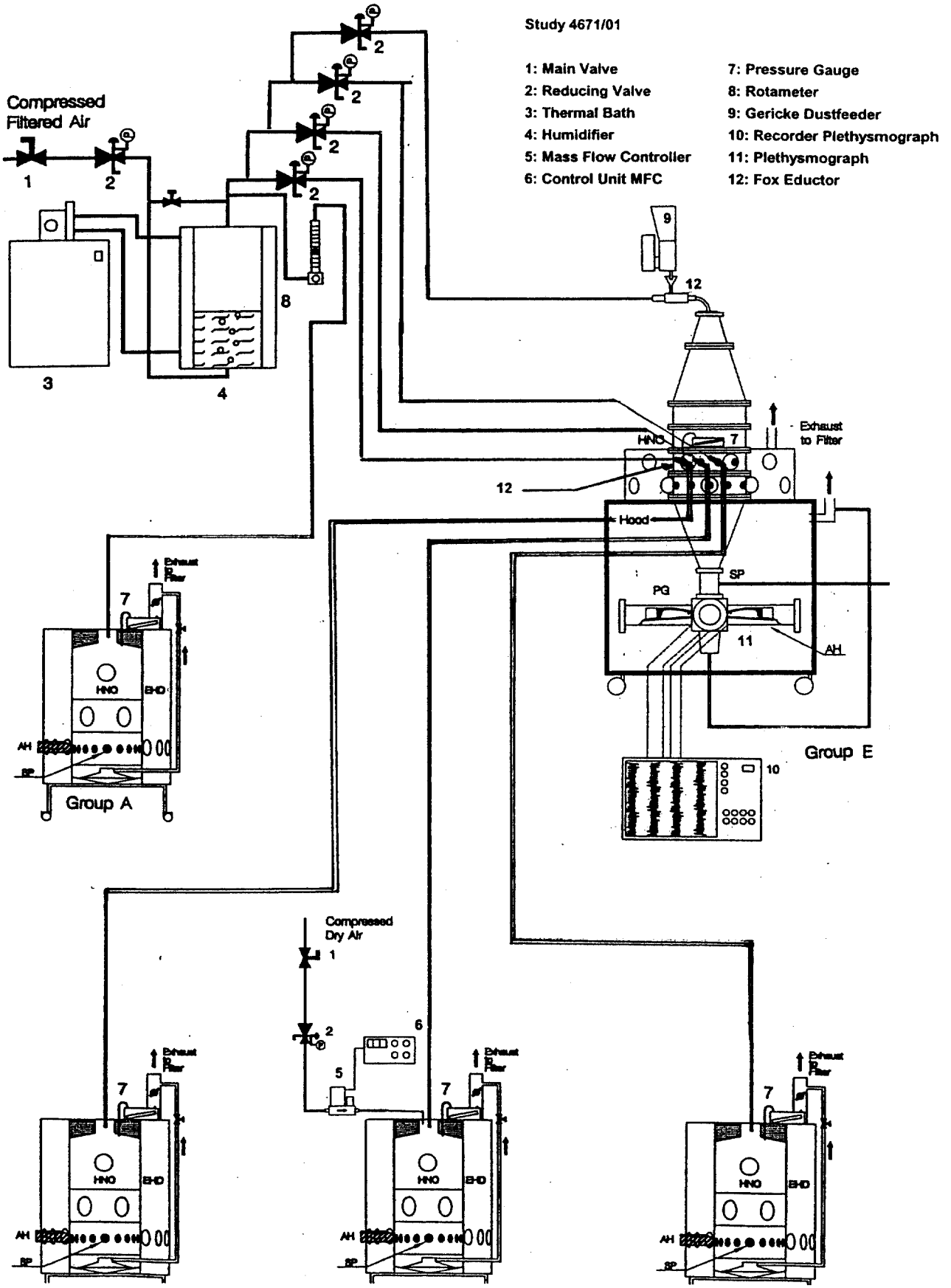

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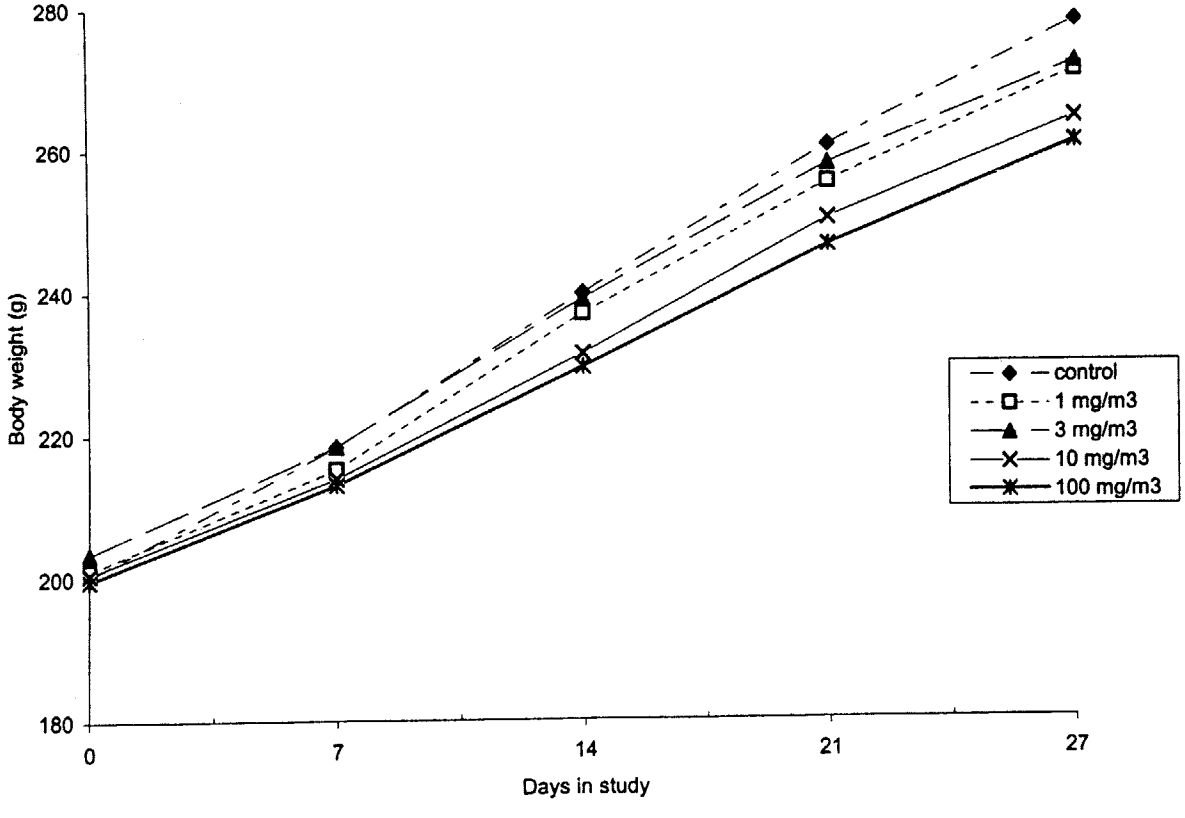
Figure 2 Mean body weights (males)



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Tables

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Table 1.1 - Actual concentration (in mg/m^3)

Date d/m	Unit B			Unit C			Unit D			Unit E		
	conc.	sd	n	conc.	sd	n	conc.	sd	n	conc.	sd	n
3/7	1.33	-	1	3.73	0.01	2	10.8	0.5	3	114.7	11.3	3
4/7	0.80	-	1	2.63	0.05	2	13.5	5.3	3	109.4	8.6	3
5/7	1.15	-	1	3.33	0.15	2	10.5	1.5	3	120.9	17.1	4
8/7	0.96	-	1	3.23	0.20	2	10.0	2.1	3	100.7	5.0	3
9/7	1.11	-	1	3.18	0.07	2	9.3	0.6	3	114.4	5.0	3
10/7	1.12	-	1	3.16	0.32	2	12.1	0.9	3	108.1	2.6	3
11/7	0.94	-	1	3.19	0.08	2	11.3	0.5	3	99.8	5.6	3
12/7	0.89	-	1	2.92	0.31	2	10.6	3.0	3	99.0	1.0	3
15/7	0.94	-	1	3.21	0.13	2	10.4	1.0	3	101.5	12.4	3
16/7	0.81	-	1	2.77	0.06	2	10.3	1.0	3	93.8	4.3	3
17/7	0.93	-	1	3.30	0.28	2	11.3	1.8	3	100.5	8.9	3
18/7	0.93	-	1	3.11	0.32	2	10.3	1.6	3	107.2	6.7	3
19/7	0.99	-	1	3.10	0.10	2	8.7	0.6	3	99.9	5.3	3
22/7	0.88	-	1	3.10	0.32	2	9.8	0.3	3	99.4	2.1	3
23/7	0.95	-	1	3.21	0.10	2	8.9	1.4	3	101.6	4.3	3
24/7	1.16	-	1	2.86	0.29	2	10.6	1.3	3	100.9	0.6	3
25/7	0.92	-	1	3.18	0.36	2	8.5	1.2	3	102.8	7.8	3
26/7	1.07	-	1	2.83	0.07	2	8.9	0.3	3	98.9	2.5	3
29/7	1.05	-	1	3.06	0.13	2	10.1	0.4	3	101.3	3.3	3
30/7	1.06	-	1	2.92	0.07	2	10.1	0.3	3	100.6	2.1	3
mean	1.00			3.10			10.3			103.8		
sd	0.13			0.24			1.2			6.6		
n	20			20			20			20		

d/m = day/month

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Table 1.2 - Air flow

Date d/m	Unit A		Unit B		Unit C			Unit D			Unit E	
	setting	flow l/min	press. bar	flow l/min	press. bar	bypass %	flow l/min	press. bar	bypass %	flow l/min	press.	flow l/min
3/7	20	27	0.80	53.5	2.3	-	35.3	1.4	-	25.4	1.2	128.1
4/7	25	33	0.70	49.7	2.15	10	38.7	1.52	-	26.8	1.2	128.1
5/7	25	33	0.74	51.3	2.1	7.5	36.9	1.79	-	29.7	1.2	128.1
8/7	25	33	0.72	50.5	2.1	13.3	39.8	1.82	-	30.0	1.2	128.1
9/7	25	33	0.73	50.9	2.1	22.5	44.4	1.88	-	30.7	1.2	128.1
10/7	25	33	0.72	50.5	2.1	27.5	46.9	1.88	-	30.7	1.2	128.1
11/7	25	33	0.72	50.5	2.1	37.5	51.9	1.83	-	30.1	1.2	128.1
12/7	25	33	0.73	50.9	2.08	37.5	51.6	1.79	-	29.7	1.2	128.1
15/7	25	33	0.75	51.6	2.05	37.5	51.3	1.78	-	29.6	1.2	128.1
16/7	25	33	0.76	52.0	2.05	37.5	51.3	1.69	-	28.6	1.2	128.1
17/7	25	33	0.76	52.0	2.05	37.5	51.3	1.6	15.0	35.1	1.19	128.1
18/7	25	33	0.77	52.4	2.05	42.5	53.8	1.6	19.2	37.2	1.2	128.1
19/7	25	33	0.79	53.2	2.05	47.5	56.3	1.6	15.8	35.5	1.2	128.1
22/7	25	33	0.80	53.5	2.1	52.5	59.4	1.62	15.0	35.3	1.19	128.1
23/7	25	33	0.82	54.3	2.1	57.5	61.9	1.65	19.2	37.7	1.2	128.1
24/7	25	33	0.85	55.4	2.1	62.5	64.4	1.66	15.0	35.8	1.2	128.1
25/7	25	33	0.87	56.2	2.11	65	65.7	1.67	12.5	34.6	1.2	128.1
26/7	25	33	0.89	57.0	2.12	65	65.8	1.68	10.0	33.5	1.2	128.1
29/7	25	33	0.92	58.1	2.13	65	65.9	1.68	10.0	33.5	1.2	128.1
30/7	25	33	0.95	59.2	2.14	65	66.0	1.68	10.0	33.5	1.2	128.1
mean		32.7		53.1			52.9			32.1		128.1
sd		1.3		2.8			10.3			3.5		0
n		20		20			20			20		20

d/m = day/month

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Table 1.3 - Nominal concentration

Date d/m	Weight feeder (g) start	Weight feeder (g) stop	Total exposure time (min)	Amount used (g)	Total exposure time (min)	Nominal conc. (mg/m^3)
3/7	nm					
4/7	1632.9		383			
5/7			382			
8/7			373			
9/7		1605.8	374	27.1	1512	140
10/7			373			
11/7			372			
12/7			375			
15/7			370			
16/7		1577.2	377	28.6	1867	120
17/7			372			
18/7			370			
19/7			370			
22/7			375			
23/7		1546.9	370	30.3	1857	127
24/7			375			
25/7			375			
26/7			370			
29/7			369			
30/7		1517.7	375	29.2	1864	122
total				115.2	7100	
mean						127
efficiency						81% #

d/m = day/month; # mean actual concentration was 103.58 mg/m^3 (including an extra exposure day on 31/7/02 (due to study 4671/02); nm= not measured

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Table 1.4 - Particle size distribution

Date d/m	Unit B		Unit C		Unit D		Unit E	
	MMAD (μm)	gsd	MMAD (μm)	gsd	MMAD (μm)	gsd	MMAD (μm)	gsd
3/7								
4/7								
5/7								
8/7								
9/7								
10/7								
11/7							2.5	1.6
12/7					1.6	1.9		
15/7								
16/7			2.2	2.0				
17/7							2.5	1.8
18/7					1.4	2.1		
19/7	1.5#	2.4#						
22/7								
23/7								
24/7							2.5	1.7
25/7					1.3	2.2		
26/7			1.8	1.9				
29/7								
30/7								
mean	na	na	2.0	2.0	1.4	2.1	2.5	1.7
sd	na	na	0.3	0.1	0.2	0.2	0	0.1
n	1	1	2	2	3	3	3	3

d/m = day/month; # sample was taken on three consecutive exposure days; na = not applicable; MMAD = Mass Median Aerodynamic Diameter; gsd = geometric standard deviation (gsd) calculated as $\sqrt{(84\%/16\%)}$

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Table 2 Summary of clinical observations

Observation Request : ANY PARAMETER, ANY CONDITION, ANY LOCATION
Requested date range: Day 0 - 28

Dose Group	A	B	C	D	E
Dose	control	1 mg/m3	3 mg/m3	10 mg/m3	100 mg/m3
Total animals	6	6	6	6	6
M A L E S					
MOUTH					
MALOCCLUSION OF INCISORS					
Incidence (%)	0(0)	0(0)	0(0)	1(16)	0(0)

In brackets: the number of animals showing the observation expressed as percentage of the total number of animals in the group

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Table 3 Mean body weights (g)

M A L E S

	control			1 mg/m3			3 mg/m3			10 mg/m3			100 mg/m3		
	Mean	sem	n	Mean	sem	n	Mean	sem	n	Mean	sem	n	Mean	sem	n
Day 0	200.3	5.7	6	201.1	7.2	6	203.5	6.7	6	200.6	3.5	6	199.7	5.5	6
Day 7	218.4	7.2	6	215.3	7.0	6	218.5	8.2	6	214.0	5.6	6	213.1	6.0	6
Day 14	239.8	8.8	6	236.9	7.5	6	239.0	8.9	6	231.3	7.8	6	229.4	6.7	6
Day 21	260.4	9.5	6	255.2	6.9	6	257.8	11.0	6	250.1	9.6	6	246.3	7.8	6
Day 27	277.8	9.6	6	270.7	7.1	6	271.9	11.4	6	264.2	9.8	6	260.7	9.6	6
grand means:	239.3			235.8			238.1			232.0			229.8		

Statistics: Analysis of Covariance + Dunnett's tests. (Two-Sided) * P<0.05 ; ** P<0.01 ; Exp.Unit = Animal

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Table 4.1 Mean food intake (g/rat/day)[1]

M A L E S

	control Mean	1 mg/m3 Mean	3 mg/m3 Mean	10 mg/m3 Mean	100 mg/m3 Mean
Day 7	19.0	18.2	18.3	17.7	17.6
Day 14	20.1	19.4	19.4	18.8	18.0
Day 21	20.8	19.7	20.4	19.8	18.9
Day 27	21.2	20.0	20.2	19.8	19.2
grand means:	20.3	19.3	19.6	19.0	18.4

[1] Food intake was measured per cage (3 rats/cage) and expressed as g/rat/day

Statistics: Anova + L.S.D. tests. (Two-Sided) * P<0.05 ; ** P<0.01 ; *** P<0.001 ; Exp.Unit = Cage Mean

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Table 4.2 Mean food conversion efficiency (g weight gain/g food consumed)[1]

M A L E S

	control Mean	1 mg/m3 Mean	3 mg/m3 Mean	10 mg/m3 Mean	100 mg/m3 Mean
Day 7	0.14	0.11	0.12	0.11	0.11
Day 14	0.15	0.16	0.15	0.13	0.13
Day 21	0.14	0.13	0.13	0.14	0.13
Day 27	0.14	0.13	0.12	0.12	0.12
grand means:	0.14	0.13	0.13	0.12	0.12

[1] Food intake was measured per cage (3 rats/cage) and expressed as g/rat/day

Statistics: Anova + L.S.D. tests. (Two-Sided) * P<0.05 ; ** P<0.01 ; *** P<0.001 ; Exp.Unit = Cage Mean

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Table 5.1 Mean haematological findings in blood collected from the abdominal aorta at the end of the treatment period (red blood cells)

M A L E S		RBC 10E12/1	HB mmol/l	PCV 1/1	MCV fl	MCH fmol	MCHC mmol/l	Reticulo /1000	Thromboc 10E9/l	PTT sec
control	Mean	7.45	9.6	0.407	54.7	1.29	23.5	48.6	1063	37.6
	sem	0.11	0.2	0.006	0.4	0.01	0.1	1.7	35	0.4
	n	6	6	6	6	6	6	6	6	6
1 mg/m3	Mean	7.34	9.5	0.407	55.5	1.29	23.3	52.2	989	37.9
	sem	0.17	0.2	0.009	0.5	0.01	0.1	2.4	19	0.8
	n	6	6	6	6	6	6	6	6	6
3 mg/m3	Mean	7.49	9.8	0.420	56.1	1.30	23.3	48.2	1000	37.3
	sem	0.09	0.1	0.007	0.4	0.01	0.2	1.9	50	0.5
	n	6	6	6	6	6	6	6	6	6
10 mg/m3	Mean	7.77	9.9	0.427	55.0	1.28	23.2	50.5	1012	37.8
	sem	0.12	0.1	0.005	0.7	0.02	0.2	2.8	25	0.9
	n	6	6	6	6	6	6	6	6	6
100 mg/m3	Mean	7.55	9.6	0.413	54.7	1.27	23.2	46.0	1031	37.8
	sem	0.10	0.1	0.004	0.6	0.01	0.1	1.6	34	1.1
	n	6	6	6	6	6	6	6	6	6

Statistics (two-sided; exp.unit = animal):
. One-way analysis of variance followed by Dunnett's multiple comparison tests; * P<0.05 ** P<0.01
In case of inhomogeneous variances (tested by means of Bartlett's test), or in case of non-continuous parameters:
. Kruskal-Wallis non-parametric analysis of variance followed by Mann-Whitney U-tests; $ P<0.05 $$ P<0.02 $$$ P<0.002

RBC = Red Blood Cells
PCV = Packed Cell Volume
MCH = Mean Corpuscular Haemoglobin
Reticulo = Reticulocytes
PTT = Prothrombin Time
HB = Haemoglobin
MCV = Mean Corpuscular Volume
MCHC = Mean Corpuscular Haemoglobin Concentration
Thromboc = Thrombocytes

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Table 5.2 Mean total and differential white blood cell counts (absolute numbers) in blood collected from the abdominal aorta at the end of the treatment period

M A L E S		WBC 10E9/1	Eosino 10E9/1	Neutro 10E9/1	Lympho 10E9/1	Mono 10E9/1	Baso 10E9/1
control	Mean	5.6	0.1	0.5	5.0	0.0	0.00
	sem	0.5	0.0	0.1	0.4	0.0	0.00
	n	6	6	6	6	6	6
1 mg/m3	Mean	5.9	0.0	0.5	5.3	0.0	0.03**
	sem	0.2	0.0	0.1	0.2	0.0	0.01
	n	6	6	6	6	6	6
3 mg/m3	Mean	6.7	0.0	0.6	6.1	0.0	0.00
	sem	0.5	0.0	0.1	0.5	0.0	0.00
	n	6	6	6	6	6	6
10 mg/m3	Mean	6.2	0.1	0.8	5.3	0.0	0.00
	sem	1.1	0.0	0.2	0.9	0.0	0.00
	n	6	6	6	6	6	6
100 mg/m3	Mean	5.7	0.0	0.8	4.9	0.0	0.00
	sem	1.2	0.0	0.2	1.0	0.0	0.00
	n	6	6	6	6	6	6

Statistics (two-sided; exp.unit = animal):
. One-way analysis of variance followed by Dunnett's multiple comparison tests; * P<0.05 ** P<0.01
In case of inhomogeneous variances (tested by means of Bartlett's test), or in case of non-continuous parameters:
. Kruskal-Wallis non-parametric analysis of variance followed by Mann-Whitney U-tests; $ P<0.05 $$ P<0.02 $$$ P<0.002

WBC = White Blood Cells
Neutro = Absolute number of Neutrophils
Mono = Absolute number of Monocytes
Eosino = Absolute number of Eosinophils
Lympho = Absolute number of Lymphocytes
Baso = Absolute number of Basophils

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Table 5.3 Mean total and differential white blood cell counts (percentages) in blood collected from the abdominal aorta at the end of the treatment period

M A L E S		WBC 10E9/l	Eosinoph %	Neutroph %	Lymphoc %	Monocyt %	Basophil %
control	Mean	5.6	1.0	9.0	89.5	0.5	0.0
	sem	0.5	0.3	1.3	1.5	0.2	0.0
	n	6	6	6	6	6	6
1 mg/m3	Mean	5.9	0.3	8.5	90.2	0.5	0.5
	sem	0.2	0.3	0.9	1.2	0.2	0.2
	n	6	6	6	6	6	6
3 mg/m3	Mean	6.7	0.3	9.0	90.7	0.0	0.0
	sem	0.5	0.2	1.0	0.8	0.0	0.0
	n	6	6	6	6	6	6
10 mg/m3	Mean	6.2	1.0	12.5	86.5	0.0	0.0
	sem	1.1	0.3	1.2	1.2	0.0	0.0
	n	6	6	6	6	6	6
100 mg/m3	Mean	5.7	1.0	14.3	84.7	0.0	0.0
	sem	1.2	0.4	2.1	2.1	0.0	0.0
	n	6	6	6	6	6	6

Statistics (two-sided; exp.unit = animal):
. One-way analysis of variance followed by Dunnett's multiple comparison tests; * P<0.05 ** P<0.01
In case of inhomogeneous variances (tested by means of Bartlett's test), or in case of non-continuous parameters:
. Kruskal-Wallis non-parametric analysis of variance followed by Mann-Whitney U-tests; $ P<0.05 $$ P<0.02 $$$ P<0.002

WBC = White Blood Cells
Eosinoph = Eosinophils
Neutroph = Neutrophils
Lymphoc = Lymphocytes
Monocyt = Monocytes
Basophil = Basophils

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Table 6.1 Mean results of clinical chemistry in plasma collected from the abdominal aorta at the end of the treatment period

M A L E S		Gluc mmol/l	ALP U/l	ALAT U/l	ASAT U/l	GGT U/l	TP g/l	Album g/l	A/G Rati
control	Mean	7.07	93	31	54	0.0	53	36	2.02
	sem	0.42	7	2	1	0.0	1	1	0.07
	n	6	6	6	6	6	6	6	6
1 mg/m3	Mean	7.37	103	30	61	0.0	53	35	2.04
	sem	0.31	6	1	3	0.0	1	1	0.04
	n	6	6	6	6	6	6	6	6
3 mg/m3	Mean	7.86	94	31	63	0.2	55	37	2.04
	sem	0.53	4	1	4	0.1	1	1	0.03
	n	6	6	6	6	6	6	6	6
10 mg/m3	Mean	7.83	95	32	62	0.1	56	36	1.84
	sem	0.35	4	2	6	0.1	0	0	0.08
	n	6	6	5	5	6	6	6	6
100 mg/m3	Mean	6.55	95	34	68	0.0	54	35	1.83
	sem	0.39	4	2	3	0.0	1	1	0.09
	n	6	6	6	5	6	6	6	6

Statistics (two-sided; exp.unit = animal):
. One-way analysis of variance followed by Dunnett's multiple comparison tests; * P<0.05 ** P<0.01
In case of inhomogeneous variances (tested by means of Bartlett's test), or in case of non-continuous parameters:
. Kruskal-Wallis non-parametric analysis of variance followed by Mann-Whitney U-tests; $ P<0.05 $$ P<0.02 $$$ P<0.002

Gluc = Glucose
ALAT = Alanine Aminotransferase (GPT)
GGT = Gamma Glutamyl Transferase
Album = Albumin
ALP = Alkaline Phosphatase
ASAT = Aspartate Aminotransferase (GOT)
TP = Total Protein
A/G Rati = Albumin/Globulin Ratio

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Table 6.2 Mean results of clinical chemistry in plasma collected from the abdominal aorta at the end of the treatment period

M A L E S		Urea mmol/l	Creatin umol/l	Bili-Tot umol/l	Cholest mmol/l	Triglyc mmol/l	Phos-lip mmol/l
control	Mean	6.8	26	1.3	1.44	0.22	1.21
	sem	0.2	1	0.9	0.04	0.02	0.01
	n	6	6	6	6	6	6
1 mg/m3	Mean	6.8	28	0.2	1.59	0.31	1.33
	sem	0.3	1	0.1	0.06	0.02	0.06
	n	6	6	6	6	6	6
3 mg/m3	Mean	6.5	27	0.6	1.61	0.31	1.36
	sem	0.3	1	0.3	0.06	0.04	0.04
	n	6	6	6	6	6	6
10 mg/m3	Mean	7.0	27	0.6	1.59	0.38	1.35
	sem	0.2	1	0.2	0.06	0.09	0.05
	n	6	6	5	6	6	6
100 mg/m3	Mean	7.1	27	0.4	1.52	0.26	1.26
	sem	0.2	1	0.2	0.04	0.03	0.03
	n	6	6	5	6	6	6

Statistics (two-sided; exp.unit = animal):
. One-way analysis of variance followed by Dunnett's multiple comparison tests; * P<0.05 ** P<0.01
In case of inhomogeneous variances (tested by means of Bartlett's test), or in case of non-continuous parameters:
. Kruskal-Wallis non-parametric analysis of variance followed by Mann-Whitney U-tests; $ P<0.05 $$ P<0.02 $$$ P<0.002

Urea = Urea in Plasma
Bili-Tot = Bilirubin (total)
Triglyc = Triglycerides
Creatin = Creatinine
Cholest = Cholesterol (total)
Phos-lip = Phospholipids

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Table 6.3 Mean results of clinical chemistry in plasma collected from the abdominal aorta at the end of the treatment period

M A L E S		Ca mmol/l	K mmol/l	Na mmol/l	Cl mmol/l	Inorg-P mmol/l
control	Mean	2.60	4.3	146	105	2.29
	sem	0.03	0.2	0	0	0.11
	n	6	6	6	6	6
1 mg/m3	Mean	2.57	4.6	145	105	2.50
	sem	0.01	0.3	0	0	0.09
	n	6	6	6	6	6
3 mg/m3	Mean	2.65	4.4	146	105	2.41
	sem	0.05	0.2	0	1	0.07
	n	6	6	6	6	6
10 mg/m3	Mean	2.53	4.9	147	104	2.47
	sem	0.05	0.4	0	1	0.16
	n	6	5	6	6	6
100 mg/m3	Mean	2.53	4.9	147	105	2.61
	sem	0.08	0.4	1	1	0.10
	n	6	6	6	6	6

Statistics (two-sided; exp.unit = animal):
. One-way analysis of variance followed by Dunnett's multiple comparison tests; * P<0.05 ** P<0.01
In case of inhomogeneous variances (tested by means of Bartlett's test), or in case of non-continuous parameters:
. Kruskal-Wallis non-parametric analysis of variance followed by Mann-Whitney U-tests; $ P<0.05 $$ P<0.02 $$$ P<0.002

Ca = Calcium
K = Potassium
Na = Sodium
Cl = Chloride
Inorg-P = Inorganic Phosphate

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Table 7.1 Mean terminal body weights (g) and absolute organ weights (g) at the end of the treatment period

M A L E S		TermBW g	Testes g	Adrenals g	Kidneys g	Brain g	Spleen g	Heart g	Liver g	Lung g
control	Mean	250.6	3.10	0.056	1.82	1.75	0.552	0.99	7.57	1.15
	sem	9.4	0.04	0.002	0.07	0.02	0.018	0.04	0.33	0.03
	n	6	6	6	6	6	6	6	6	5
1 mg/m3	Mean	245.1	2.99	0.065	1.62	1.75	0.589	0.95	7.17	1.29
	sem	7.0	0.10	0.004	0.06	0.03	0.040	0.02	0.23	0.05
	n	6	6	6	6	6	6	6	6	6
3 mg/m3	Mean	244.6	2.97	0.060	1.67	1.75	0.527	0.97	7.17	1.47**
	sem	10.4	0.12	0.002	0.07	0.01	0.029	0.04	0.40	0.06
	n	6	6	6	6	6	6	6	6	6
10 mg/m3	Mean	236.9	2.71	0.059	1.64	1.71	0.497	0.92	7.03	1.77**
	sem	8.9	0.18	0.003	0.06	0.01	0.028	0.05	0.31	0.05
	n	6	6	6	6	6	6	6	6	6
100 mg/m3	Mean	234.4	2.77	0.056	1.59	1.69	0.510	0.95	7.00	2.02**
	sem	8.4	0.14	0.003	0.06	0.04	0.038	0.05	0.27	0.04
	n	6	6	6	6	6	6	6	6	6

TermBW = Terminal Body Weight

Statistics (two-sided; exp.unit = animal):
. One-way analysis of variance followed by Dunnett's multiple comparison tests; * P<0.05 ** P<0.01
In case of inhomogeneous variances (tested by means of Bartlett's test), or in case of non-continuous parameters:
. Kruskal-Wallis non-parametric analysis of variance followed by Mann-Whitney U-tests; $ P<0.05 $$ P<0.02 $$$ P<0.002

	n	6	6	6	6	6	6	6	6	6
10 mg/m3	Mean	236.9	11.43	0.249	6.94	7.24	2.10	3.87	29.7	7.48**
	sem	8.9	0.56	0.009	0.20	0.25	0.09	0.08	0.7	0.19
	n	6	6	6	6	6	6	6	6	6
100 mg/m3	Mean	234.4	11.82	0.241	6.79	7.24	2.18	4.03	29.9	8.69**
	sem	8.4	0.46	0.019	0.16	0.17	0.16	0.17	0.7	0.43
	n	6	6	6	6	6	6	6	6	6

TermBW = Terminal Body Weight

Statistics (two-sided; exp.unit = animal):
. One-way analysis of variance followed by Dunnett's multiple comparison tests; * P<0.05 ** P<0.01
In case of inhomogeneous variances (tested by means of Bartlett's test), or in case of non-continuous parameters:
. Kruskal-Wallis non-parametric analysis of variance followed by Mann-Whitney U-tests; $ P<0.05 $$ P<0.02 $$$ P<0.002

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Table 8: Summary of macroscopic observations the day after the last exposure

CHANGES	TREATMENT	INCIDENCE OF LESIONS (NUMERIC) Males				
		Contr.	1 mg/m3	3 mg/m3	10 mg/m3	100 mg/m3
KIDNEYS						
Uni-lateral flabby		1				
Uni-lateral hydronephrosis					1	
TESTES						
Uni-lateral cryptorchism					1	
Uni-lateral small					1	
THORACIC CAVITY						
Enlarged parathymic lymphnodes						1

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Table 9: Summary of microscopic observations the day after the last exposure

CHANGES	TREATMENT	INCIDENCE OF LESIONS (NUMERIC) Males				
		Contr.	1 mg/m3	3 mg/m3	10 mg/m3	100 mg/m3
KIDNEYS		(1)				
Basophilic tubules		1				
Focal mineralisation		1				
Transitional cell hyperplasia		1				
Bi-lateral hydronephrosis		1				
LARYNX		(6)	(6)	(5)	(6)	(6)
Focal granulomatous inflammation		0	0	0	0	3
Focal subepithelial necrosis		0	0	0	0	2
Focal subepithelial mineralisation		0	0	0	0	2
Focal squamous metaplasia		2	1	2	4	3
Epithelial microcyst(s)		0	0	0	0	1
Mononuclear cell infiltrate		5	2	4	4	0*
Lymphoid aggregates		2	1	1	0	0
Focal laryngitis		1	0	0	0	0

Statistics:2-sided Fisher's exact test between the controls & each of the treatment groups. *P<0.05, **P<0.01, ***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically
Low numbers in brackets, representing the microscopic verification of gross observations,
were not subjected to statistical evaluation
Study : 4671/01

Report Continued....

NOCOLOK flux 28-day inhalation toxicity study in rats
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Study: 4671/01
Table 9: Summary of microscopic observations the day after the last exposure

CHANGES	TREATMENT	INCIDENCE OF LESIONS (NUMERIC) Males				
		Contr.	1 mg/m3	3 mg/m3	10 mg/m3	100 mg/m3
LUNGS		(6)	(6)	(6)	(6)	(6)
Large alveolar macrophages						
very slight		0	6**	2	0	0
slight		0	0	4	5*	3
moderate		0	0	0	1	3
Score Expanded Totals		0	6**	6**	6**	6**
Alveolar proteinosis		0	0	0	0	3
Perivascular mononuclear cell infiltrate						
very slight		0	0	3	2	2
slight		0	0	1	2	2
moderate		0	0	0	1	2
Score Expanded Totals		0	0	4	5*	6**
BALT germinal centre development		0	1	3	3	5*
(Focal) alveolitis						
very slight		0	0	1	2	3
slight		0	0	1	0	3
Score Expanded Totals		0	0	2	2	6**
Bronchial/bronchiolar epithelial hypertrophy						
slight		0	0	3	1	1
Score Expanded Totals		0	0	3	1	1
Accumulation of alveolar macrophages		1	0	0	0	0

Statistics:2-sided Fisher's exact test between the controls & each of the treatment groups. *P<0.05, **P<0.01, ***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically
Low numbers in brackets, representing the microscopic verification of gross observations,
were not subjected to statistical evaluation

Report Continued....

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Table 9: Summary of microscopic observations the day after the last exposure

CHANGES	TREATMENT	INCIDENCE OF LESIONS (NUMERIC) Males				
		Contr.	1 mg/m3	3 mg/m3	10 mg/m3	100 mg/m3
LUNGS		(6)	(6)	(6)	(6)	(6)
Bone spherule/spicule		0	0	0	1	1
NASAL CAVITY		(6)	(5)	(6)	(6)	(6)
Focal olfactory epithelial necrosis						
very slight		0	0	1	0	0
slight		0	0	0	0	1
moderate		0	0	0	1	5*
Score Expanded Totals		0	0	1	1	6**
Focal olfactory epithelial vacuolation						
very slight		0	0	0	1	0
slight		0	0	1	1	0
Score Expanded Totals		0	0	1	2	0
(Focal) respiratory epithelial metaplasia						
slight		0	0	0	0	5*
moderate		0	0	0	0	1
Score Expanded Totals		0	0	0	0	6**
(Focal) goblet cell hyperplasia						
very slight		0	0	0	1	6**
slight		0	0	0	1	0
Score Expanded Totals		0	0	0	2	6**
Focal respiratory epithelial mineral deposit(s)		5	2	4	4	6

Statistics:2-sided Fisher's exact test between the controls & each of the treatment groups. *P<0.05, **P<0.01, ***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically
Low numbers in brackets, representing the microscopic verification of gross observations,
were not subjected to statistical evaluation
Study : 4671/01

Report Continued....

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Table 9: Summary of microscopic observations the day after the last exposure

CHANGES	TREATMENT	INCIDENCE OF LESIONS (NUMERIC) Males				
		Contr.	1 mg/m3	3 mg/m3	10 mg/m3	100 mg/m3
THORACIC CAVITY						(1)
Macrophage aggregate(s) parathymic lymphnodes						1
TRACHEA/BRONCHI		(6)				(6)
No abnormality detected		6				6

Statistics:2-sided Fisher's exact test between the controls & each of the treatment groups. *P<0.05, **P<0.01, ***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically
Low numbers in brackets, representing the microscopic verification of gross observations,
were not subjected to statistical evaluation
Study : 4671/01

Report Complete.

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Annexes

Annex 1

TNO Nutrition and Food Research

C R O S S R E F E R E N C E L I S T I N G

Study : 4671/01 NOMINAL DAY ZERO 03-JUL-02

MALES

GROUP	CAGE	ANM_ID
A	2	2
A	2	4
A	2	6
A	4	8
A	4	10
A	4	12
B	6	14
B	6	16
B	6	18
B	8	20
B	8	22
B	8	24
C	10	26
C	10	28
C	10	30
C	12	32
C	12	34
C	12	36
D	14	38
D	14	40
D	14	42
D	16	44
D	16	46
D	16	48
E	18	50
E	18	52
E	18	54
E	20	56
E	20	58
E	20	60

TOTAL MALES = 30

CAGE = CAGE NUMBER ANM_ID = ANIMAL IDENTIFICATION NUMBER

Annex 2, Certificate of analysis of the diet used

Special Diets Services

Special Quality Control Certificate of Analysis

PRODUCT: RM3 (E) SQC FG BATCH NO: 2206

PREMIX BATCH NO: 575 DATE OF MANUFACTURE: 09-APR-02

Nutrient	Found Analysis		Contaminant	Found Analysis		Limit of Detectio
Moisture	10.2	%	Fluoride	23	mg/kg	1.0 mg/k
Crude Fat	5.3	%	Nitrate as NaNO3	18	mg/kg	1.0 mg/k
Crude Protein	22.5	%	Nitrite as NaNO2	Non Detected	mg/kg	1.0 mg/k
Crude Fibre	5.3	%	Lead	Non Detected	mg/kg	0.25 mg/k
Ash	6.8	%	Arsenic	Non Detected	mg/kg	0.2 mg/k
Calcium	1.20	%	Cadmium	0.05	mg/kg	0.05 mg/k
Phosphorus	0.87	%	Mercury	0.02	mg/kg	0.01 mg/k
Sodium	0.32	%	Selenium	0.42	mg/kg	0.05 mg/k
Chloride	0.56	%				
Potassium	0.97	%				
Magnesium	0.25	%	Total Aflatoxins	Non Detected	mcg/kg	1 mcg/kg each of B1,B2,G1,C
Iron	201	mg/kg				
Copper	12	mg/kg	Total P.C.B	Non Detected	mcg/kg	10.0 mcg/k
Manganese	89	mg/kg	Total D.D.T	Non Detected	mcg/kg	10.0 mcg/k
Zinc	58	mg/kg	Dieldrin	Non Detected	mcg/kg	10.0 mcg/k
			Lindane	Non Detected	mcg/kg	10.0 mcg/k
			Heptachlor	Non Detected	mcg/kg	10.0 mcg/k
			Malathion	Non Detected	mcg/kg	20.0 mcg/k
Vitamin A	15.0	iu/g	Total Viable Organisms x 1000	Non Detected	per grm	1000/g
Vitamin E	74	mg/kg				
Vitamin C		mg/kg	Mesophilic Spores x 100	Non Detected	per grm	100/g
			Salmonellae Species	Non Detected	per grm	Absent in 20 grm
			Entero Bacteriaceae	Non Detected	per grm	Absent in 20 grm
			Escherichia Coli	Non Detected	per grm	Absent in 20 grm
			Fungal Units	20	per grm	Absent in 20 grm
			Antibiotic Activity	Non Detected		

Signed .....

Dated 3/5/2002

Annex 3, Parameters checked in drinking water

Results of periodical analyses in drinking water collected on the premises of TNO Nutrition and Food Research in Zeist, the Netherlands.

This is a translation of the Analysis Report of N.V. Hydron Midden-Nederland, dated 19 July 2002.
The analyses were conducted in samples taken on 3 July 2002, in room number 05.1.11 at TNO Nutrition and Food Research, Utrechtseweg 48, Zeist.

Parameter	Unit	Measured
Odour (qualitative)		odourless
Clarity (qualitative)		clear
Oxygen	mg/l	9.05
pH		7.85
Taste (qualitative)		good
Temperature	°C	18
Non Purgeable Organic Carbon	mg C/l	0.29
Iron	mg/l	0.036
Electrical conductivity	mS/m	24.2
Manganese	mg/l	<0.002
Ammonia	mg N/l	<0.03
Nitrite	mg N/l	<0.002
Nitrate	mg N/l	1.04
Cadmium	µg/l	<0.4
Copper	µg/l	120
Lead	µg/l	<3
Aeromonas bacteria	#/100 ml	<10
Coli bacteria (37°C)	#/100 ml	<1
Plate count 22°C	#/ml	10
Plate count 37°C	#/ml	1

Annex 4 Listing of haematology parameters and methods of analysis

Parameter	Method	Reference
Haemoglobin	Sysmex K-1000 Haematology Analyzer. Toa Medical Electronics Co., Ltd., Japan CiHb measurement	Manufacturer's manual (1988) Based on Helleman, P.W. et al., Haematology. Elsevier, Amsterdam, the Netherlands, 1973, p.33
Packed cell volume	Sysmex K-1000 Haematology Analyzer. Toa Medical Electronics Co., Ltd., Japan	Manufacturer's manual (1988) Based on cumulative pulse height detection
Red blood cell count	Sysmex K-1000 Haematology Analyzer. Toa Medical Electronics Co., Ltd., Japan	Manufacturer's manual (1988) Based on electric resistance detection
Total white blood cell count	Sysmex K-1000 Haematology Analyzer. Toa Medical Electronics Co., Ltd., Japan	Manufacturer's manual (1988) Based on electric resistance detection
Differential white blood cell count	Microscopic examination of stained blood smears according to Pappenheim. Absolute numbers are calculated from total white blood cells and percentage distribution of each cell type	Gorter, E. and W.C. de Graaff, Klinische Diagnostiek, 7th ed., H.E. Stenfert Kroese N.V., Leiden, the Netherlands, 1955, part I, p. 34
Reticulocytes	Microscopic examination of blood smears stained with new methylene blue	Helleman, P.W. et al., Haematologie. Elsevier, Amsterdam, the Netherlands, 1973, p.49
Prothrombin time	Normotest, modified method for EDTA blood Nyegaard and Co. A/S, Oslo, Norway	Manufacturer's manual based on Owren, P.A. (1969) Pharmakotherapi 25
Thrombocyte count	Sysmex K-1000 Haematology Analyzer. Toa Medical Electronics Co., Ltd., Japan	Manufacturer's manual Based on electric resistance detection
Mean corpuscular volume (MCV)	Calculated $MCV = \frac{packed\ cell\ volume}{red\ blood\ cells}$	
Mean corpuscular haemoglobin (MCH)	Calculated $MCH = \frac{haemoglobin}{red\ blood\ cells}$	
Mean corpuscular haemoglobin concentration (MCHC)	Calculated $MCHC = \frac{haemoglobin}{packed\ cell\ volume}$	

Annex 5 Listing of clinical chemistry parameters and methods of analysis

Parameter	Method	Reference
Glucose	Hitachi-911 analyzer Hexokinase, Boehringer reagent	Manufacturer's manual
Alkaline phosphatase activity (ALP)	Hitachi-911 analyzer Boehringer reagent	Manufacturer's manual according to to I.F.C.C. Based on Tietz, N.W. et al. (1983) *J.Clin.Chem.Clin. Biochem.* **21,** 731-748
Alanine aminotransferase (ALAT)/ glutamic-pyruvic transaminase (GPT) activity	Hitachi-911 analyzer Boehringer reagent	Manufacturer's manual according to I.F.C.C. without PLP. Based on Bergmeyer, H.U. et al.(1986) *J.Clin.Chem.Clin.Biochem.* **24,** 481
Aspartate aminotransferase (ASAT)/ glutamic-oxalacetic transaminase (GOT) activity	Hitachi-911 analyzer Boehringer reagent	Manufacturer's manual according to I.F.C.C. without PLP. Based on Bergmeyer, H.U. et al. (1986) *J.Clin.Chem.Clin.Biochem.* **24,** 497
γ-Glutamyl transferase activity (GGT)	Hitachi-911 analyzer Boehringer reagent	Manufacturer's manual Based on Szasz, G. et al. (1974) *Z.Klin.Chem.Klin.Biochem.* **12,** 228
Total protein	Hitachi-911 analyzer Boehringer reagent Biuret	Manufacturer's manual Based on Weichselbaum, T.E. (1946) *Am.J.Clin.Path.* **16,** 40
Albumin	Hitachi-911 analyzer Boehringer reagent Bromcresol green	Manufacturer's manual Based on Doumas, B.T. et al. (1971) *Clin.Chim.Acta* **31,** 87
Ratio albumin to globulin	Calculated, $ratio = \frac{albumin}{total\ protein - albumin}$	
Urea	Hitachi-911 analyzer Boehringer reagent Urease-UV	Manufacturer's manual Based on Neumann, U. et al. (1977) *Scand.J.Clin.Lab.Invest.* **37,** suppl. 147, abstract 97
Creatinine	Hitachi-911 analyzer Boehringer reagent Enzymatic PAP	Manufacturer's manual Based on Siedel, J. et al. (1984) *Clin.Chem.* **30,** 968

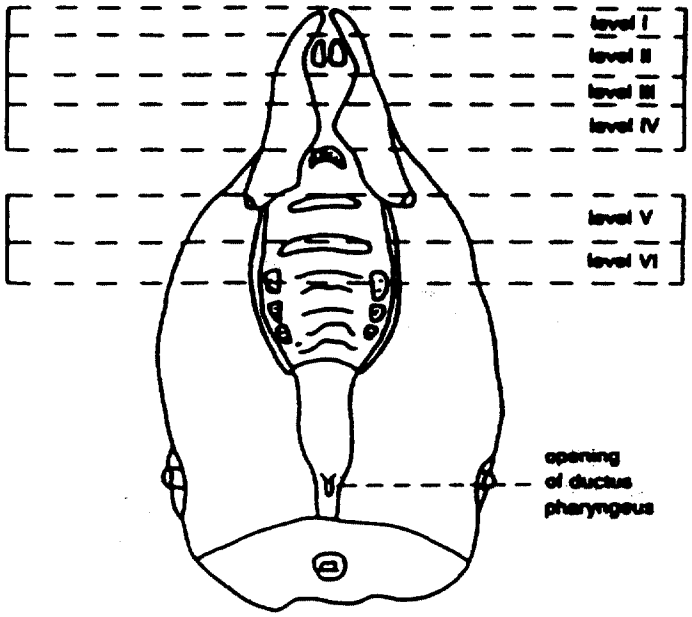
I.F.C.C. = International Federation of Clinical Chemistry
PLP = pyridoxalphosphate
PAP = phenol-4-aminophenazone

Annex 5 (cont.) Listing of clinical chemistry parameters parameters and methods of analysis

Parameter	Method	Reference
Bilirubin (total)	Hitachi-911 analyzer Boehringer reagent Diazotized sulphanilic acid	Manufacturer's manual Based on Jendrassik, L. et al. (1938) *Biochem.Z.* **297**, 81
Cholesterol (total)	Hitachi-911 analyzer Boehringer reagent CHOD-PAP	Manufacturer's manual Based on Siedel, J. et al. (1983) *Clin.Chem.* **29**, 1075
Triglycerides	Hitachi-911 analyzer Boehringer reagent Enzymatic GPO-PAP	Manufacturer's manual Based on Bergmeyer, H.U. (1974) Methoden der enzymatischen Analyse, Auflage 3
Phospholipids	Hitachi-911 analyzer Boehringer reagent Enzymatic	Manufacturer's manual Based on Takayama, M. et al. (1977) *Clin.Chim.Acta* **79**, 93-98
Calcium (Ca)	Hitachi-911 analyzer Boehringer reagent o-Cresolphthalein-Komplexon	Manufacturer's manual Based on Gindler, E.M. et al. (1972) *Am.J.Clin.Pathol.* **59**, 836
Sodium (Na)	Hitachi-911 analyzer Boehringer reagent I.S.E.	Manufacturer's manual Ion Selective Electrode (I.S.E.)
Potassium (K)	Hitachi-911 analyzer Boehringer reagent I.S.E.	Manufacturer's manual Ion Selective Electrode
Chloride (Cl)	Hitachi-911 analyzer Boehringer reagent I.S.E.	Manufacturer's manual Ion Selective Electrode
Inorganic phosphate	Hitachi-911 analyzer Boehringer reagent Molybdate-UV	Manufacturer's manual Based on Henry, R.J. (1974). Clinical Chemistry. Harper & Row Publishers, New York

CHOD-PAP = cholesterol oxidase - phenol-4-aminophenazone
GPO-PAP = glycerolphosphate oxidase - phenol-4-aminophenazone

Annex 6 - Schematic indication of the cross sections through the nose (Woutersen et al., 1994)



Ventral view of the rat hard palate region with the lower jaw removed, indicating the six standard cross sections through the nose (I to VI).

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Appendices

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Appendix 1.1 - Temperature (°C) during exposure

Date d/m	Unit A		Unit B		Unit C		Unit D		Unit E	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
3/7#	21.5	0.1	21.7	0	21.8	0.3	22.0	0.2	22.4	0
4/7	21.4	0.4	21.7	0.4	22.2	0.2	22.2	0.4	22.3	0.3
5/7	21.3	0.5	21.6	0.4	21.9	0.3	21.8	0.4	22.0	0.2
8/7	22.4	0.1	22.3	0.1	22.3	0.1	22.4	0.1	22.8	0.2
9/7	22.5	0.1	22.4	0.1	22.4	0.1	22.5	0.1	22.8	0.1
10/7	22.0	0.2	22.2	0.2	22.4	0.6	22.5	0.3	23.3	0.2
11/7	21.8	0.4	21.9	0.4	22.3	0.3	22.4	0.3	22.8	0.3
12/7	21.9	0.4	21.9	0.3	22.3	0.3	22.3	0.2	22.9	0.3
15/7	21.8	0.4	21.9	0.4	22.2	0.4	22.4	0.4	22.7	0.4
16/7	22.1	0.4	22.2	0.3	22.4	0.3	22.6	0.3	23.1	0.4
17/7	22.1	0.3	22.3	0.3	22.6	0.3	23.1	0.2	23.0	0.2
18/7	21.9	0.5	22.1	0.5	22.3	0.6	22.5	0.6	22.7	0.6
19/7	22.0	0.4	22.1	0.3	22.4	0.3	22.7	0.4	22.8	0.3
22/7	21.3	0.5	21.4	0.5	21.7	0.5	21.9	0.6	22.1	0.4
23/7	22.1	0.2	22.2	0.2	22.5	0.1	22.9	0.1	23.0	0.1
24/7	21.9	0.2	22.1	0.2	22.4	0.2	22.5	0.1	22.8	0.1
25/7	21.7	0.3	21.9	0.3	22.2	0.3	22.4	0.3	22.6	0.3
26/7	21.8	0.4	22.1	0.4	22.3	0.4	22.3	0.4	22.6	0.6
29/7	22.0	0.3	22.1	0.3	22.4	0.4	22.6	0.5	22.8	0.4
30/7	22.5	0.4	22.8	0.1	23.1	0.3	23.2	0.2	23.3	0.4
mean	21.9		22.1		22.3		22.5		22.7	
sd	0.3		0.3		0.3		0.4		0.3	
n	20		20		20		20		20	

d/m = day/month; # only measured twice instead of 3 times/day

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Appendix 1.2 - Relative humidity (RH) during exposure

Date d/m	Unit A		Unit B		Unit C		Unit D		Unit E	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
3/7#	31.4	1.3	31.1	1.2	34.7	0.8	35.1	0.1	44.0	0.4
4/7	33.9	1.7	32.8	1.2	38.0	3.5	38.3	2.5	40.2	2.1
5/7	32.8	1.8	32.6	1.5	35.4	2.2	35.9	2.5	36.0	2.7
8/7	33.3	0.5	33.2	0.3	33.0	0.4	35.2	0.8	42.1	0.9
9/7	33.1	0.4	32.8	0.8	32.7	0.5	35.9	0.5	41.7	0.1
10/7	32.5	0.4	32.6	0.5	32.2	2.4	34.2	1.7	40.1	0.5
11/7	33.8	1.5	32.4	1.0	35.2	1.5	37.9	1.5	41.9	2.0
12/7	33.8	0.7	32.6	1.2	35.4	1.2	37.0	0.8	40.0	0.8
15/7	32.0	0.7	32.6	0.6	35.0	0.7	36.9	1.3	41.1	0.7
16/7	31.6	0.6	31.7	1.3	33.6	1.1	36.1	0.2	38.8	0.8
17/7	31.2	0.7	31.7	0.8	34.5	0.8	39.3	2.9	42.6	0.5
18/7	32.0	1.0	32.7	1.1	33.5	1.3	35.2	0.9	42.1	0.3
19/7	32.4	1.7	32.7	1.5	34.8	1.1	35.4	1.1	37.8	0.8
22/7	33.0	1.1	33.6	0.9	35.3	1.0	37.2	0.9	40.5	0.8
23/7	31.6	0.5	32.1	0.7	33.9	0.7	36.2	1.7	39.8	2.0
24/7	31.8	0.6	32.9	1.1	34.3	1.5	36.4	1.1	43.2	0.6
25/7	32.3	0.7	33.3	0.8	35.2	1.0	36.5	1.1	40.0	0.2
26/7	32.2	0.7	33.4	0.7	34.8	0.5	34.1	0.6	42.0	1.1
29/7	32.2	1.0	32.9	1.3	34.4	1.7	34.6	0.9	43.3	1.0
30/7	31.0	0.7	31.7	0.4	32.0	0.5	33.3	0.3	43.3	1.5
mean	32.4		32.6		34.4		36.0		41.0	
sd	0.9		0.6		1.4		1.5		2.0	
n	20		20		20		20		20	

d/m = day/month; # only measured twice instead of 3 times/day

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Appendix 2 Individual clinical observations

Observation Request : ANY PARAMETER, ANY CONDITION, ANY LOCATION

Animal	Observation	Days (Requested date range: day 0 to day 28)	
Group: A	Dose: control		
2	KILLED (SCHEDULED)	28	(31-JUL-02)
4	KILLED (SCHEDULED)	28	(31-JUL-02)
6	KILLED (SCHEDULED)	28	(31-JUL-02)
8	KILLED (SCHEDULED)	28	(31-JUL-02)
10	KILLED (SCHEDULED)	28	(31-JUL-02)
12	KILLED (SCHEDULED)	28	(31-JUL-02)
Group: B	Dose: 1 mg/m3		
14	KILLED (SCHEDULED)	28	(31-JUL-02)
16	KILLED (SCHEDULED)	28	(31-JUL-02)
18	KILLED (SCHEDULED)	28	(31-JUL-02)
20	KILLED (SCHEDULED)	28	(31-JUL-02)
22	KILLED (SCHEDULED)	28	(31-JUL-02)
24	KILLED (SCHEDULED)	28	(31-JUL-02)
Group: C	Dose: 3 mg/m3		
26	KILLED (SCHEDULED)	28	(31-JUL-02)
28	KILLED (SCHEDULED)	28	(31-JUL-02)
30	KILLED (SCHEDULED)	28	(31-JUL-02)
32	KILLED (SCHEDULED)	28	(31-JUL-02)
34	KILLED (SCHEDULED)	28	(31-JUL-02)
36	KILLED (SCHEDULED)	28	(31-JUL-02)

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Appendix 2 Individual clinical observations

Observation Request : ANY PARAMETER, ANY CONDITION, ANY LOCATION

Animal	Observation	Days (Requested date range: day 0 to day 28)	
Group: D	Dose: 10 mg/m3		
38	KILLED (SCHEDULED)	28	(31-JUL-02)
40	KILLED (SCHEDULED)	28	(31-JUL-02)
42	KILLED (SCHEDULED)	28	(31-JUL-02)
44	KILLED (SCHEDULED)	28	(31-JUL-02)
46	KILLED (SCHEDULED)	28	(31-JUL-02)
	MOUTH: MALOCCLUSION OF INCISORS	3-6	
48	KILLED (SCHEDULED)	28	(31-JUL-02)
Group: E	Dose: 100 mg/m3		
50	KILLED (SCHEDULED)	28	(31-JUL-02)
52	KILLED (SCHEDULED)	28	(31-JUL-02)
54	KILLED (SCHEDULED)	28	(31-JUL-02)
56	KILLED (SCHEDULED)	28	(31-JUL-02)
58	KILLED (SCHEDULED)	28	(31-JUL-02)
60	KILLED (SCHEDULED)	28	(31-JUL-02)

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Appendix 3 Individual body weights (g)

M A L E S		Animal No.	Day 0	Day 7	Day 14	Day 21	Day 27
control	A	2	215.7	240.8	269.8	293.0	306.8
	A	4	193.6	203.3	218.2	235.2	247.2
	A	6	209.7	229.8	251.4	271.4	294.0
	A	8	198.8	220.7	242.7	264.3	281.8
	A	10	177.0	192.5	211.7	231.7	252.0
	A	12	206.9	223.5	244.8	266.6	285.2
control		Mean	200.3	218.4	239.8	260.4	277.8
		sem	5.7	7.2	8.8	9.5	9.6
		n	6	6	6	6	6
1 mg/m3	B	14	226.4	231.8	251.6	263.0	272.4
	B	16	188.1	199.9	221.9	240.1	261.1
	B	18	212.7	224.5	246.3	263.5	284.2
	B	20	197.7	224.7	251.1	272.1	284.4
	B	22	176.5	188.5	207.0	228.6	240.2
	B	24	205.0	222.4	243.4	263.7	281.9
1 mg/m3		Mean	201.1	215.3	236.9	255.2	270.7
		sem	7.2	7.0	7.5	6.9	7.1
		n	6	6	6	6	6
3 mg/m3	C	26	228.4	241.5	262.9	281.6	293.6
	C	28	193.8	207.1	222.5	234.0	248.8
	C	30	212.5	239.8	266.1	296.0	313.8
	C	32	201.0	219.3	239.1	257.4	270.1
	C	34	180.1	188.8	211.6	226.8	238.6
	C	36	205.4	214.2	231.5	251.1	266.4
3 mg/m3		Mean	203.5	218.5	239.0	257.8	271.9
		sem	6.7	8.2	8.9	11.0	11.4
		n	6	6	6	6	6

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Appendix 3 Individual body weights (g)

M A L E S		Animal No.	Day 0	Day 7	Day 14	Day 21	Day 27
10 mg/m3	D	38	211.6	237.7	265.6	294.8	310.8
	D	40	193.3	207.4	217.6	240.5	255.2
	D	42	206.6	220.0	237.7	250.2	262.5
	D	44	198.1	210.5	228.9	248.7	263.3
	D	46	189.2	197.7	212.1	226.2	242.3
	D	48	204.8	210.7	225.7	240.0	251.2
10 mg/m3		Mean	200.6	214.0	231.3	250.1	264.2
		sem	3.5	5.6	7.8	9.6	9.8
		n	6	6	6	6	6
100 mg/m3	E	50	212.3	229.6	245.9	260.1	272.9
	E	52	191.9	206.3	225.2	242.1	254.9
	E	54	209.5	231.2	251.7	274.7	297.9
	E	56	204.5	204.2	213.1	222.6	229.9
	E	58	176.5	194.2	212.5	230.9	245.6
	E	60	203.7	213.2	227.9	247.5	262.8
100 mg/m3		Mean	199.7	213.1	229.4	246.3	260.7
		sem	5.5	6.0	6.7	7.8	9.6
		n	6	6	6	6	6

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Appendix 4.1 Individual haematological findings in blood collected from the abdominal aorta at the end of the treatment period (red blood cells)

M A L E S		Animal No.	RBC 10E12/l	HB mmol/l	PCV l/l	MCV fl	MCH fmol	MCHC mmol/l	Reticulo /1000	Thromboc 10E9/l	PTT sec
control	A	2	7.70	10.2	0.427	55.5	1.32	23.9	41.3	1026	39.0
	A	4	7.73	9.8	0.409	52.9	1.27	24.0	51.6	1052	36.0
	A	6	7.53	9.7	0.412	54.7	1.29	23.5	48.2	1227	37.5
	A	8	7.17	9.2	0.392	54.7	1.28	23.5	47.8	1077	37.5
	A	10	7.45	9.6	0.412	55.3	1.29	23.3	53.5	990	37.6
	A	12	7.11	9.0	0.391	55.0	1.27	23.0	49.2	1003	37.7
control		Mean	7.45	9.6	0.407	54.7	1.29	23.5	48.6	1063	37.6
		sem	0.11	0.2	0.006	0.4	0.01	0.1	1.7	35	0.4
		n	6	6	6	6	6	6	6	6	6
1 mg/m3	B	14	7.52	9.8	0.415	55.2	1.30	23.6	50.7	1007	39.4
	B	16	7.71	9.8	0.419	54.3	1.27	23.4	56.3	1029	34.6
	B	18	7.64	9.9	0.424	55.5	1.30	23.3	56.3	973	37.7
	B	20	6.55	8.5	0.362	55.3	1.30	23.5	59.0	971	37.9
	B	22	7.39	9.3	0.405	54.8	1.26	23.0	47.0	1040	40.2
	B	24	7.20	9.6	0.415	57.6	1.33	23.1	44.0	911	37.4
1 mg/m3		Mean	7.34	9.5	0.407	55.5	1.29	23.3	52.2	989	37.9
		sem	0.17	0.2	0.009	0.5	0.01	0.1	2.4	19	0.8
		n	6	6	6	6	6	6	6	6	6
3 mg/m3	C	26	7.52	9.6	0.409	54.4	1.28	23.5	50.8	843	38.2
	C	28	7.58	9.9	0.429	56.6	1.31	23.1	49.5	921	36.4
	C	30	7.30	9.5	0.411	56.3	1.30	23.1	55.0	1178	37.2
	C	32	7.71	10.3	0.440	57.1	1.34	23.4	46.6	1030	39.1
	C	34	7.68	9.8	0.433	56.4	1.28	22.6	41.2	941	35.7
	C	36	7.14	9.5	0.398	55.7	1.33	23.9	46.0	1088	37.1
3 mg/m3		Mean	7.49	9.8	0.420	56.1	1.30	23.3	48.2	1000	37.3
		sem	0.09	0.1	0.007	0.4	0.01	0.2	1.9	50	0.5
		n	6	6	6	6	6	6	6	6	6

RBC = Red Blood Cells
PCV = Packed Cell Volume
MCH = Mean Corpuscular Haemoglobin
Reticulo = Reticulocytes
PTT = Prothrombin Time
HB = Haemoglobin
MCV = Mean Corpuscular Volume
MCHC = Mean Corpuscular Haemoglobin Concentration
Thromboc = Thrombocytes

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Appendix 4.1 Individual haematological findings in blood collected from the abdominal aorta at the end of the treatment period (red blood cells)

M A L E S		Animal No.	RBC 10E12/l	HB mmol/l	PCV 1/l	MCV fl	MCH fmol	MCHC mmol/l	Reticulo /1000	Thromboc 10E9/l	PTT sec
10 mg/m3	D	38	7.36	9.8	0.410	55.7	1.33	23.9	63.5	1084	39.7
	D	40	7.82	9.8	0.421	53.8	1.25	23.3	50.3	936	40.2
	D	42	7.75	9.7	0.426	55.0	1.25	22.8	47.3	944	35.7
	D	44	7.72	10.1	0.446	57.8	1.31	22.6	44.9	1068	39.2
	D	46	7.69	9.9	0.424	55.1	1.29	23.3	50.7	1034	37.1
	D	48	8.25	10.1	0.435	52.7	1.22	23.2	46.2	1004	34.9
10 mg/m3		Mean	7.77	9.9	0.427	55.0	1.28	23.2	50.5	1012	37.8
		sem	0.12	0.1	0.005	0.7	0.02	0.2	2.8	25	0.9
		n	6	6	6	6	6	6	6	6	6
100 mg/m3	E	50	7.69	9.6	0.412	53.6	1.25	23.3	50.4	1072	34.4
	E	52	7.37	9.4	0.404	54.8	1.28	23.3	40.6	1099	40.0
	E	54	7.47	9.5	0.412	55.2	1.27	23.1	50.3	1090	36.4
	E	56	7.91	10.0	0.431	54.5	1.26	23.2	43.3	912	41.3
	E	58	7.61	9.5	0.404	53.1	1.25	23.5	47.3	1078	38.7
	E	60	7.24	9.5	0.415	57.3	1.31	22.9	44.2	937	36.1
100 mg/m3		Mean	7.55	9.6	0.413	54.7	1.27	23.2	46.0	1031	37.8
		sem	0.10	0.1	0.004	0.6	0.01	0.1	1.6	34	1.1
		n	6	6	6	6	6	6	6	6	6

RBC = Red Blood Cells
PCV = Packed Cell Volume
MCH = Mean Corpuscular Haemoglobin
Reticulo = Reticulocytes
PTT = Prothrombin Time
HB = Haemoglobin
MCV = Mean Corpuscular Volume
MCHC = Mean Corpuscular Haemoglobin Concentration
Thromboc = Thrombocytes

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Appendix 4.2 Individual total and differential white blood cell counts (absolute numbers) in blood collected from the abdominal aorta at the end of the treatment period

M A L E S		Animal No.	WBC 10E9/l	Eosino 10E9/l	Neutro 10E9/l	Lympho 10E9/l	Mono 10E9/l	Baso 10E9/l
control	A	2	6.4	0.1	0.8	5.4	0.1	0.0
	A	4	7.0	0.0	0.4	6.6	0.0	0.0
	A	6	5.7	0.1	0.7	4.9	0.1	0.0
	A	8	5.2	0.1	0.4	4.8	0.0	0.0
	A	10	3.7	0.1	0.2	3.4	0.0	0.0
	A	12	5.5	0.1	0.6	4.8	0.1	0.0
control		Mean	5.6	0.1	0.5	5.0	0.0	0.0
		sem	0.5	0.0	0.1	0.4	0.0	0.0
		n	6	6	6	6	6	6
1 mg/m3	B	14	6.5	0.1	0.6	5.7	0.1	0.1
	B	16	6.0	0.0	0.6	5.4	0.0	0.0
	B	18	5.3	0.0	0.5	4.8	0.0	0.1
	B	20	5.4	0.0	0.5	4.8	0.1	0.0
	B	22	5.4	0.0	0.5	4.8	0.1	0.1
	B	24	6.5	0.0	0.3	6.2	0.0	0.0
1 mg/m3		Mean	5.9	0.0	0.5	5.3	0.0	0.0
		sem	0.2	0.0	0.1	0.2	0.0	0.0
		n	6	6	6	6	6	6
3 mg/m3	C	26	6.5	0.0	0.7	5.8	0.0	0.0
	C	28	5.7	0.0	0.6	5.1	0.0	0.0
	C	30	6.6	0.1	0.4	6.1	0.0	0.0
	C	32	6.6	0.1	0.4	6.1	0.0	0.0
	C	34	6.0	0.0	0.7	5.3	0.0	0.0
	C	36	9.0	0.0	0.8	8.2	0.0	0.0
3 mg/m3		Mean	6.7	0.0	0.6	6.1	0.0	0.0
		sem	0.5	0.0	0.1	0.5	0.0	0.0
		n	6	6	6	6	6	6

WBC = White Blood Cells
Neutro = Absolute number of Neutrophils
Mono = Absolute number of Monocytes
Eosino = Absolute number of Eosinophils
Lympho = Absolute number of Lymphocytes
Baso = Absolute number of Basophils

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Appendix 4.2 Individual total and differential white blood cell counts (absolute numbers) in blood collected from the abdominal aorta at the end of the treatment period

M A L E S		Animal No.	WBC 10E9/l	Eosino 10E9/l	Neutro 10E9/l	Lympho 10E9/l	Mono 10E9/l	Baso 10E9/l
10 mg/m3	D	38	6.2	0.1	0.6	5.6	0.0	0.0
	D	40	8.2	0.0	1.2	7.0	0.0	0.0
	D	42	2.5	0.0	0.2	2.3	0.0	0.0
	D	44	6.3	0.1	0.9	5.3	0.0	0.0
	D	46	3.9	0.0	0.5	3.4	0.0	0.0
	D	48	10.0	0.2	1.5	8.3	0.0	0.0
10 mg/m3		Mean	6.2	0.1	0.8	5.3	0.0	0.0
		sem	1.1	0.0	0.2	0.9	0.0	0.0
		n	6	6	6	6	6	6
100 mg/m3	E	50	8.8	0.0	1.8	7.0	0.0	0.0
	E	52	9.5	0.0	0.9	8.6	0.0	0.0
	E	54	4.2	0.1	0.6	3.5	0.0	0.0
	E	56	5.8	0.1	0.5	5.2	0.0	0.0
	E	58	2.8	0.1	0.6	2.2	0.0	0.0
	E	60	3.1	0.0	0.4	2.7	0.0	0.0
100 mg/m3		Mean	5.7	0.0	0.8	4.9	0.0	0.0
		sem	1.2	0.0	0.2	1.0	0.0	0.0
		n	6	6	6	6	6	6

WBC = White Blood Cells
Neutro = Absolute number of Neutrophils
Mono = Absolute number of Monocytes
Eosino = Absolute number of Eosinophils
Lympho = Absolute number of Lymphocytes
Baso = Absolute number of Basophils

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Appendix 4.3 Individual total and differential white blood cell counts (percentages) in blood collected from the abdominal aorta at the end of the treatment period

M A L E S		Animal No.	WBC 10E9/l	Eosinoph %	Neutroph %	Lymphoc %	Monocyt %	Basophil %
control	A	2	6.4	1.0	13.0	85.0	1.0	0.0
	A	4	7.0	0.0	6.0	94.0	0.0	0.0
	A	6	5.7	1.0	12.0	86.0	1.0	0.0
	A	8	5.2	1.0	7.0	92.0	0.0	0.0
	A	10	3.7	2.0	6.0	92.0	0.0	0.0
	A	12	5.5	1.0	10.0	88.0	1.0	0.0
control		Mean	5.6	1.0	9.0	89.5	0.5	0.0
		sem	0.5	0.3	1.3	1.5	0.2	0.0
		n	6	6	6	6	6	6
1 mg/m3	B	14	6.5	2.0	9.0	87.0	1.0	1.0
	B	16	6.0	0.0	10.0	90.0	0.0	0.0
	B	18	5.3	0.0	9.0	90.0	0.0	1.0
	B	20	5.4	0.0	10.0	89.0	1.0	0.0
	B	22	5.4	0.0	9.0	89.0	1.0	1.0
	B	24	6.5	0.0	4.0	96.0	0.0	0.0
1 mg/m3		Mean	5.9	0.3	8.5	90.2	0.5	0.5
		sem	0.2	0.3	0.9	1.2	0.2	0.2
		n	6	6	6	6	6	6
3 mg/m3	C	26	6.5	0.0	11.0	89.0	0.0	0.0
	C	28	5.7	0.0	11.0	89.0	0.0	0.0
	C	30	6.6	1.0	6.0	93.0	0.0	0.0
	C	32	6.6	1.0	6.0	93.0	0.0	0.0
	C	34	6.0	0.0	11.0	89.0	0.0	0.0
	C	36	9.0	0.0	9.0	91.0	0.0	0.0
3 mg/m3		Mean	6.7	0.3	9.0	90.7	0.0	0.0
		sem	0.5	0.2	1.0	0.8	0.0	0.0
		n	6	6	6	6	6	6

WBC = White Blood Cells
Neutroph = Neutrophils
Monocyt = Monocytes
Eosinoph = Eosinophils
Lymphoc = Lymphocytes
Basophil = Basophils

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Appendix 4.3 Individual total and differential white blood cell counts (percentages) in blood collected from the abdominal aorta at the end of the treatment period

M A L E S		Animal No.	WBC 10E9/l	Eosinoph %	Neutroph %	Lymphoc %	Monocyt %	Basophil %
10 mg/m3	D	38	6.2	1.0	9.0	90.0	0.0	0.0
	D	40	8.2	0.0	15.0	85.0	0.0	0.0
	D	42	2.5	1.0	9.0	90.0	0.0	0.0
	D	44	6.3	1.0	15.0	84.0	0.0	0.0
	D	46	3.9	1.0	12.0	87.0	0.0	0.0
	D	48	10.0	2.0	15.0	83.0	0.0	0.0
10 mg/m3		Mean	6.2	1.0	12.5	86.5	0.0	0.0
		sem	1.1	0.3	1.2	1.2	0.0	0.0
		n	6	6	6	6	6	6
100 mg/m3	E	50	8.8	0.0	21.0	79.0	0.0	0.0
	E	52	9.5	0.0	10.0	90.0	0.0	0.0
	E	54	4.2	2.0	14.0	84.0	0.0	0.0
	E	56	5.8	2.0	9.0	89.0	0.0	0.0
	E	58	2.8	2.0	20.0	78.0	0.0	0.0
	E	60	3.1	0.0	12.0	88.0	0.0	0.0
100 mg/m3		Mean	5.7	1.0	14.3	84.7	0.0	0.0
		sem	1.2	0.4	2.1	2.1	0.0	0.0
		n	6	6	6	6	6	6

WBC = White Blood Cells
Neutroph = Neutrophils
Monocyt = Monocytes
Eosinoph = Eosinophils
Lymphoc = Lymphocytes
Basophil = Basophils

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Appendix 5.1 Individual results of clinial chemistry in plasma collected from the abdominal aorta at the end of the treatment period

M A L E S		Animal No.	Gluc mmol/l	ALP U/l	ALAT U/l	ASAT U/l	GGT U/l	TP g/l	Album g/l	A/G Rati
control	A	2	7.69	92	34	57	0.0	55	36	1.89
	A	4	8.19	68	35	52	0.0	54	38	2.38
	A	6	7.79	102	33	54	0.0	53	35	1.94
	A	8	5.44	85	31	56	0.0	51	34	2.00
	A	10	6.88	118	27	55	0.0	54	36	2.00
	A	12	6.40	90	26	50	0.0	52	34	1.89
control		Mean	7.07	93	31	54	0.0	53	36	2.02
		sem	0.42	7	2	1	0.0	1	1	0.07
		n	6	6	6	6	6	6	6	6
1 mg/m3	B	14	8.78	85	31	63	0.0	56	37	1.95
	B	16	7.37	115	26	60	0.0	55	37	2.06
	B	18	7.09	89	33	57	0.0	55	38	2.24
	B	20	6.60	94	30	55	0.0	48	32	2.00
	B	22	7.45	120	27	56	0.0	51	34	2.00
	B	24	6.93	116	35	77	0.0	51	34	2.00
1 mg/m3		Mean	7.37	103	30	61	0.0	53	35	2.04
		sem	0.31	6	1	3	0.0	1	1	0.04
		n	6	6	6	6	6	6	6	6
3 mg/m3	C	26	6.85	101	31	80	0.0	51	34	2.00
	C	28	7.71	85	37	69	0.0	54	36	2.00
	C	30	9.27	88	29	55	0.9	56	37	1.95
	C	32	9.62	108	31	54	0.0	56	38	2.11
	C	34	7.26	82	30	57	0.2	56	38	2.11
	C	36	6.46	101	26	61	0.0	55	37	2.06
3 mg/m3		Mean	7.86	94	31	63	0.2	55	37	2.04
		sem	0.53	4	1	4	0.1	1	1	0.03
		n	6	6	6	6	6	6	6	6

Gluc = Glucose
ALAT = Alanine Aminotransferase (GPT)
GGT = Gamma Glutamyl Transferase
Album = Albumin

ALP = Alkaline Phosphatase
ASAT = Aspartate Aminotransferase (GOT)
TP = Total Protein
A/G Rati = Albumin/Globulin Ratio

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Appendix 5.1 Individual results of clinial chemistry in plasma collected from the abdominal aorta at the end of the treatment period

M A L E S		Animal No.	Gluc mmol/l	ALP U/l	ALAT U/l	ASAT U/l	GGT U/l	TP g/l	Album g/l	A/G Rati
10 mg/m3	D	38	7.19	112	35	58	0.0	57	36	1.71
	D	40	7.98	86	33	54	0.0	54	37	2.18
	D	42	7.59	93	38	87	0.0	56	36	1.80
	D	44	8.80	87	31	54	0.5	56	36	1.80
	D	46	6.66	103	NM	NM	0.0	55	34	1.62
	D	48	8.74	90	24	56	0.0	56	37	1.95
10 mg/m3		Mean	7.83	95	32	62	0.1	56	36	1.84
		sem	0.35	4	2	6	0.1	0	0	0.08
		n	6	6	5	5	6	6	6	6
100 mg/m3	E	50	8.13	98	38	69	0.0	56	37	1.95
	E	52	6.15	109	35	77	0.0	54	35	1.84
	E	54	6.15	84	29	59	0.0	56	36	1.80
	E	56	7.20	83	30	70	0.0	55	36	1.89
	E	58	5.52	99	40	NM	0.0	53	31	1.41
	E	60	6.13	97	32	67	0.0	52	35	2.06
100 mg/m3		Mean	6.55	95	34	68	0.0	54	35	1.83
		sem	0.39	4	2	3	0.0	1	1	0.09
		n	6	6	6	5	6	6	6	6

NM = Not Measured; due to haemolytic sample

Gluc = Glucose
ALAT = Alanine Aminotransferase (GPT)
GGT = Gamma Glutamyl Transferase
Album = Albumin
ALP = Alkaline Phosphatase
ASAT = Aspartate Aminotransferase (GOT)
TP = Total Protein
A/G Rati = Albumin/Globulin Ratio

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Appendix 5.2 Individual results of clinial chemistry in plasma collected from the abdominal aorta at the end of the treatment period

M A L E S		Animal No.	Urea mmol/l	Creatin umol/l	Bili-Tot umol/l	Cholest mmol/l	Triglyc mmol/l	Phos-lip mmol/l
control	A	2	7.1	28	0.9	1.40	0.27	1.22
	A	4	6.1	22	5.5	1.27	0.22	1.20
	A	6	6.4	27	0.9	1.46	0.22	1.21
	A	8	6.9	28	0.4	1.44	0.13	1.18
	A	10	6.3	26	0.0	1.51	0.23	1.25
	A	12	7.7	25	0.1	1.53	0.24	1.20
control		Mean	6.8	26	1.3	1.44	0.22	1.21
		sem	0.2	1	0.9	0.04	0.02	0.01
		n	6	6	6	6	6	6
1 mg/m3	B	14	5.6	28	0.3	1.84	0.37	1.55
	B	16	7.6	31	0.3	1.66	0.26	1.42
	B	18	6.6	26	0.4	1.59	0.28	1.28
	B	20	6.6	31	0.0	1.40	0.35	1.18
	B	22	7.6	25	0.0	1.50	0.34	1.24
	B	24	6.6	25	0.0	1.55	0.28	1.28
1 mg/m3		Mean	6.8	28	0.2	1.59	0.31	1.33
		sem	0.3	1	0.1	0.06	0.02	0.06
		n	6	6	6	6	6	6
3 mg/m3	C	26	6.1	27	0.0	1.46	0.24	1.29
	C	28	6.6	26	0.2	1.73	0.28	1.50
	C	30	6.4	25	1.6	1.41	0.36	1.23
	C	32	7.6	28	0.4	1.56	0.45	1.39
	C	34	6.7	29	1.1	1.81	0.37	1.45
	C	36	5.8	25	0.2	1.67	0.18	1.30
3 mg/m3		Mean	6.5	27	0.6	1.61	0.31	1.36
		sem	0.3	1	0.3	0.06	0.04	0.04
		n	6	6	6	6	6	6

Urea = Urea in Plasma
Bili-Tot = Bilirubin (total)
Triglyc = Triglycerides
Creatin = Creatinine
Cholest = Cholesterol (total)
Phos-lip = Phospholipids

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Appendix 5.2 Individual results of clinial chemistry in plasma collected from the abdominal aorta at the end of the treatment period

M A L E S		Animal No.	Urea mmol/l	Creatin umol/l	Bili-Tot umol/l	Cholest mmol/l	Triglyc mmol/l	Phos-lip mmol/l
10 mg/m3	D	38	7.1	29	0.8	1.72	0.16	1.31
	D	40	6.0	26	1.0	1.47	0.27	1.32
	D	42	7.0	27	0.0	1.52	0.26	1.32
	D	44	7.3	23	0.6	1.79	0.77	1.57
	D	46	7.1	28	NM	1.52	0.46	1.26
	D	48	7.5	27	0.4	1.49	0.35	1.31
10 mg/m3		Mean	7.0	27	0.6	1.59	0.38	1.35
		sem	0.2	1	0.2	0.06	0.09	0.05
		n	6	6	5	6	6	6
100 mg/m3	E	50	7.6	28	0.5	1.52	0.37	1.30
	E	52	7.0	27	0.7	1.60	0.24	1.31
	E	54	6.5	29	1.0	1.55	0.34	1.34
	E	56	7.3	26	0.0	1.47	0.22	1.26
	E	58	7.4	26	NM	1.64	0.16	1.20
	E	60	6.9	26	0.0	1.33	0.20	1.17
100 mg/m3		Mean	7.1	27	0.4	1.52	0.26	1.26
		sem	0.2	1	0.2	0.04	0.03	0.03
		n	6	6	5	6	6	6

NM = Not Measured; due to haemolytic sample

Urea = Urea in Plasma
Bili-Tot = Bilirubin (total)
Triglyc = Triglycerides
Creatin = Creatinine
Cholest = Cholesterol (total)
Phos-lip = Phospholipids

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Appendix 5.3 Individual results of clinial chemistry in plasma collected from the abdominal aorta at the end of the treatment period

M A L E S		Animal No.	Ca mmol/l	K mmol/l	Na mmol/l	Cl mmol/l	Inorg-P mmol/l
control	A	2	2.57	4.1	144	105	1.90
	A	4	2.55	3.9	145	106	2.07
	A	6	2.66	4.2	146	105	2.32
	A	8	2.67	4.7	146	105	2.59
	A	10	2.51	4.9	147	104	2.54
	A	12	2.66	4.1	145	104	2.32
control		Mean	2.60	4.3	146	105	2.29
		sem	0.03	0.2	0	0	0.11
		n	6	6	6	6	6
1 mg/m3	B	14	2.55	5.3	146	105	2.51
	B	16	2.57	5.0	146	105	2.46
	B	18	2.62	3.6	145	106	2.14
	B	20	2.58	4.7	145	105	2.66
	B	22	2.55	4.0	144	105	2.49
	B	24	2.54	4.7	145	105	2.75
1 mg/m3		Mean	2.57	4.6	145	105	2.50
		sem	0.01	0.3	0	0	0.09
		n	6	6	6	6	6
3 mg/m3	C	26	2.45	3.7	145	107	2.15
	C	28	2.58	5.0	147	105	2.44
	C	30	2.72	4.1	145	104	2.34
	C	32	2.75	4.6	145	102	2.62
	C	34	2.64	4.7	147	106	2.32
	C	36	2.77	4.4	145	106	2.61
3 mg/m3		Mean	2.65	4.4	146	105	2.41
		sem	0.05	0.2	0	1	0.07
		n	6	6	6	6	6

Ca = Calcium
Na = Sodium
Inorg-P = Inorganic Phosphate
K = Potassium
Cl = Chloride

NOCOLOK flux 28-day inhalation toxicity study in rats
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Appendix 5.3 Individual results of clinial chemistry in plasma collected from the abdominal aorta at the end of the treatment period

M A L E S		Animal No.	Ca mmol/l	K mmol/l	Na mmol/l	Cl mmol/l	Inorg-P mmol/l
10 mg/m3	D	38	2.61	4.3	148	106	2.15
	D	40	2.50	3.8	146	107	1.92
	D	42	2.48	6.4	146	104	2.76
	D	44	2.72	4.8	147	103	2.79
	D	46	2.40	NM	147	103	2.89
	D	48	2.48	5.1	145	103	2.31
10 mg/m3		Mean	2.53	4.9	147	104	2.47
		sem	0.05	0.4	0	1	0.16
		n	6	5	6	6	6
100 mg/m3	E	50	2.64	4.0	146	106	2.60
	E	52	2.76	3.7	146	107	3.10
	E	54	2.58	4.5	150	106	2.59
	E	56	2.24	4.7	148	105	2.42
	E	58	2.36	6.7	147	105	2.49
	E	60	2.57	5.5	145	103	2.44
100 mg/m3		Mean	2.53	4.9	147	105	2.61
		sem	0.08	0.4	1	1	0.10
		n	6	6	6	6	6

NM = Not Measured; due to haemolytic sample

Ca = Calcium
Na = Sodium
Inorg-P = Inorganic Phosphate
K = Potassium
Cl = Chloride

NOCOLOK flux 28-day inhalation toxicity study in rats
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Appendix 6.1 Individual terminal body weights (g) and absolute organ weights (g) at the end of the treatment period

M A L E S		Animal No.	TermBW g	Testes g	Adrenals g	Kidneys g	Brain g	Spleen g	Heart g	Liver g	Lung g
control	A	2	279.7	3.18	0.059	1.99	1.73	0.592	1.04	8.60	NM
	A	4	221.8	3.10	0.055	1.79	1.76	0.484	0.94	6.79	1.12
	A	6	264.8	2.90	0.058	1.82	1.82	0.562	1.11	7.49	1.27
	A	8	256.5	3.12	0.063	1.80	1.76	0.604	0.99	7.82	1.16
	A	10	224.4	3.19	0.051	1.52	1.70	0.538	0.82	6.53	1.08
	A	12	256.1	3.13	0.050	1.99	1.74	0.530	1.06	8.19	1.13
control		Mean	250.6	3.10	0.056	1.82	1.75	0.552	0.99	7.57	1.15
		sem	9.4	0.04	0.002	0.07	0.02	0.018	0.04	0.33	0.03
		n	6	6	6	6	6	6	6	6	5
1 mg/m3	B	14	243.7	2.75	0.061	1.56	1.77	0.507	0.93	7.69	1.41
	B	16	232.3	2.96	0.072	1.45	1.67	0.563	0.92	6.59	1.29
	B	18	257.8	3.32	0.077	1.81	1.84	0.604	0.99	7.09	1.33
	B	20	260.5	3.27	0.058	1.72	1.79	0.726	0.90	7.85	1.19
	B	22	218.2	2.95	0.051	1.51	1.67	0.463	0.95	6.43	1.12
	B	24	258.3	2.71	0.069	1.67	1.76	0.671	1.01	7.35	1.37
1 mg/m3		Mean	245.1	2.99	0.065	1.62	1.75	0.589	0.95	7.17	1.29
		sem	7.0	0.10	0.004	0.06	0.03	0.040	0.02	0.23	0.05
		n	6	6	6	6	6	6	6	6	6
3 mg/m3	C	26	264.6	3.35	0.062	1.84	1.81	0.595	1.08	7.30	1.65
	C	28	223.4	2.61	0.062	1.61	1.71	0.461	0.86	6.85	1.40
	C	30	283.1	3.20	0.058	1.94	1.77	0.595	1.08	9.03	1.60
	C	32	241.0	3.09	0.066	1.58	1.73	0.435	0.95	7.01	1.38
	C	34	215.4	2.79	0.055	1.54	1.75	0.502	0.92	6.35	1.28
	C	36	240.1	2.78	0.057	1.53	1.75	0.574	0.94	6.49	1.49
3 mg/m3		Mean	244.6	2.97	0.060	1.67	1.75	0.527	0.97	7.17	1.47
		sem	10.4	0.12	0.002	0.07	0.01	0.029	0.04	0.40	0.06
		n	6	6	6	6	6	6	6	6	6

TermBW = Terminal Body Weight
NM = Not Measured; Lungs were by mistake already inflated with formalin

NOCOLOK flux 28-day inhalation toxicity study in rats
TNO Nutrition and Food Research
Study: 4671/01

Appendix 6.1 Individual terminal body weights (g) and absolute organ weights (g) at the end of the treatment period

M A L E S		Animal No.	TermBW g	Testes g	Adrenals g	Kidneys g	Brain g	Spleen g	Heart g	Liver g	Lung g
10 mg/m3	D	38	278.6	3.18	0.068	1.77	1.72	0.606	1.15	8.12	2.00
	D	40	226.0	2.68	0.055	1.47	1.69	0.534	0.84	6.22	1.73
	D	42	236.5	2.82	0.066	1.68	1.66	0.508	0.90	7.41	1.83
	D	44	238.1	2.98	0.050	1.82	1.75	0.406	0.96	7.41	1.60
	D	46	217.6	1.90	0.056	1.59	1.72	0.450	0.85	6.75	1.74
	D	48	224.6	2.73	0.058	1.51	1.70	0.476	0.81	6.27	1.72
10 mg/m3		Mean	236.9	2.71	0.059	1.64	1.71	0.497	0.92	7.03	1.77
		sem	8.9	0.18	0.003	0.06	0.01	0.028	0.05	0.31	0.05
		n	6	6	6	6	6	6	6	6	6
100 mg/m3	E	50	245.8	2.95	0.060	1.73	1.74	0.470	0.90	7.14	1.96
	E	52	230.7	3.00	0.050	1.42	1.69	0.628	0.90	6.51	2.02
	E	54	266.5	3.05	0.056	1.77	1.84	0.504	1.21	8.24	2.05
	E	56	206.7	2.56	0.069	1.50	1.66	0.356	0.93	6.74	2.16
	E	58	220.6	2.16	0.051	1.56	1.52	0.524	0.90	6.54	2.05
	E	60	236.2	2.90	0.050	1.55	1.70	0.576	0.84	6.81	1.88
100 mg/m3		Mean	234.4	2.77	0.056	1.59	1.69	0.510	0.95	7.00	2.02
		sem	8.4	0.14	0.003	0.06	0.04	0.038	0.05	0.27	0.04
		n	6	6	6	6	6	6	6	6	6

TermBW = Terminal Body Weight

NOCOLOK flux 28-day inhalation toxicity study in rats
TNO Nutrition and Food Research
Study: 4671/01

Appendix 6.2 Individual terminal body weights (g) and relative organ weights (g/kg) at the end of the treatment period

M A L E S		Animal No.	TermBW g	Testes g/kg BW	Adrenals g/kg BW	Kidneys g/kg BW	Brain g/kg BW	Spleen g/kg BW	Heart g/kg BW	Liver g/kg BW	Lung g/kg BW
control	A	2	279.7	11.36	0.211	7.12	6.18	2.12	3.74	30.8	NM
	A	4	221.8	13.98	0.248	8.05	7.93	2.18	4.22	30.6	5.07
	A	6	264.8	10.97	0.219	6.88	6.88	2.12	4.19	28.3	4.81
	A	8	256.5	12.15	0.246	7.02	6.87	2.35	3.86	30.5	4.54
	A	10	224.4	14.23	0.227	6.79	7.57	2.40	3.66	29.1	4.81
	A	12	256.1	12.21	0.195	7.75	6.79	2.07	4.12	32.0	4.41
control		Mean	250.6	12.48	0.224	7.27	7.04	2.21	3.96	30.2	4.73
		sem	9.4	0.55	0.008	0.21	0.25	0.06	0.10	0.5	0.12
		n	6	6	6	6	6	6	6	6	5
1 mg/m3	B	14	243.7	11.29	0.250	6.41	7.26	2.08	3.81	31.6	5.80
	B	16	232.3	12.74	0.310	6.24	7.17	2.42	3.96	28.4	5.55
	B	18	257.8	12.86	0.299	7.01	7.12	2.34	3.84	27.5	5.15
	B	20	260.5	12.56	0.223	6.60	6.88	2.79	3.47	30.1	4.58
	B	22	218.2	13.52	0.234	6.91	7.68	2.12	4.34	29.5	5.12
	B	24	258.3	10.51	0.267	6.46	6.81	2.60	3.93	28.5	5.30
1 mg/m3		Mean	245.1	12.25	0.264	6.61	7.15	2.39	3.89	29.2	5.25
		sem	7.0	0.46	0.014	0.12	0.13	0.11	0.12	0.6	0.17
		n	6	6	6	6	6	6	6	6	6
3 mg/m3	C	26	264.6	12.66	0.234	6.95	6.84	2.25	4.08	27.6	6.23
	C	28	223.4	11.70	0.278	7.20	7.65	2.06	3.85	30.7	6.25
	C	30	283.1	11.31	0.205	6.85	6.24	2.10	3.82	31.9	5.66
	C	32	241.0	12.83	0.274	6.54	7.18	1.80	3.94	29.1	5.74
	C	34	215.4	12.93	0.255	7.17	8.14	2.33	4.25	29.5	5.94
	C	36	240.1	11.60	0.237	6.37	7.30	2.39	3.93	27.0	6.21
3 mg/m3		Mean	244.6	12.17	0.247	6.85	7.22	2.16	3.98	29.3	6.01
		sem	10.4	0.29	0.011	0.14	0.27	0.09	0.07	0.7	0.11
		n	6	6	6	6	6	6	6	6	6

TermBW = Terminal Body Weight
NM = Not Measured; Lungs were by mistake already inflated with formalin

NOCOLOK flux 28-day inhalation toxicity study in rats
TNO Nutrition and Food Research
Study: 4671/01

Appendix 6.2 Individual terminal body weights (g) and relative organ weights (g/kg) at the end of the treatment period

M A L E S		Animal No.	TermBW g	Testes g/kg BW	Adrenals g/kg BW	Kidneys g/kg BW	Brain g/kg BW	Spleen g/kg BW	Heart g/kg BW	Liver g/kg BW	Lung g/kg BW
10 mg/m3	D	38	278.6	11.40	0.244	6.36	6.16	2.18	4.12	29.1	7.16
	D	40	226.0	11.85	0.243	6.52	7.47	2.36	3.73	27.5	7.67
	D	42	236.5	11.91	0.279	7.10	7.03	2.15	3.81	31.3	7.73
	D	44	238.1	12.52	0.210	7.62	7.36	1.71	4.05	31.1	6.71
	D	46	217.6	8.74	0.257	7.31	7.90	2.07	3.89	31.0	7.97
	D	48	224.6	12.16	0.258	6.72	7.55	2.12	3.63	27.9	7.65
10 mg/m3		Mean	236.9	11.43	0.249	6.94	7.24	2.10	3.87	29.7	7.48
		sem	8.9	0.56	0.009	0.20	0.25	0.09	0.08	0.7	0.19
		n	6	6	6	6	6	6	6	6	6
100 mg/m3	E	50	245.8	12.00	0.244	7.04	7.10	1.91	3.67	29.1	7.98
	E	52	230.7	13.00	0.217	6.17	7.32	2.72	3.90	28.2	8.76
	E	54	266.5	11.46	0.210	6.65	6.90	1.89	4.52	30.9	7.68
	E	56	206.7	12.39	0.334	7.25	8.03	1.72	4.49	32.6	10.46
	E	58	220.6	9.78	0.231	7.07	6.89	2.38	4.08	29.7	9.29
	E	60	236.2	12.29	0.212	6.57	7.21	2.44	3.54	28.8	7.94
100 mg/m3		Mean	234.4	11.82	0.241	6.79	7.24	2.18	4.03	29.9	8.69
		sem	8.4	0.46	0.019	0.16	0.17	0.16	0.17	0.7	0.43
		n	6	6	6	6	6	6	6	6	6

TermBW = Terminal Body Weight

Study:4671/01

Animal	Group:A	Contr.	Males

A0002 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
NASAL CAVITY : Very slight focal respiratory epithelial mineral deposit(s)

NO ABNORMALITIES DETECTED IN:
LARYNX, LUNGS, TRACHEA/BRONCHI

A0004 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Slight mononuclear cell infiltrate
Lymphoid aggregates
Very slight focal laryngitis , ventral diverticulum
NASAL CAVITY : Very slight focal respiratory epithelial mineral deposit(s)

NO ABNORMALITIES DETECTED IN:
LUNGS, TRACHEA/BRONCHI

A0006 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Very slight mononuclear cell infiltrate
NASAL CAVITY : Very slight focal respiratory epithelial mineral deposit(s)

NO ABNORMALITIES DETECTED IN:
LUNGS, TRACHEA/BRONCHI

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal	Group:A	Contr.	Males

A0008 survivor
killed on day 28

Macroscopic findings
KIDNEYS : Uni-lateral flabby

Microscopic findings
KIDNEYS : Bi-lateral hydronephrosis
Slight transitional cell hyperplasia
Slight focal mineralisation
Slight basophilic tubules
LARYNX : Very slight mononuclear cell infiltrate
NASAL CAVITY : Very slight focal respiratory epithelial mineral deposit(s)

NO ABNORMALITIES DETECTED IN:
LUNGS, TRACHEA/BRONCHI

A0010 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Slight mononuclear cell infiltrate
Very slight focal squamous metaplasia
LUNGS : Very slight accumulation of alveolar macrophages

NO ABNORMALITIES DETECTED IN:
NASAL CAVITY, TRACHEA/BRONCHI

A0012 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Slight focal squamous metaplasia
Slight mononuclear cell infiltrate
Lymphoid aggregates
NASAL CAVITY : Slight focal respiratory epithelial mineral deposit(s)

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal	Group:A	Contr.	Males

A0012 Continued....

Microscopic findings
NO ABNORMALITIES DETECTED IN:
LUNGS, TRACHEA/BRONCHI

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal Group:B 1mg/m3 Males

B0014 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Lymphoid aggregates
Very slight focal squamous metaplasia
Very slight mononuclear cell infiltrate
LUNGS : Very slight large alveolar macrophages

NO ABNORMALITIES DETECTED IN:
NASAL CAVITY

B0016 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LUNGS : Very slight large alveolar macrophages
NASAL CAVITY : Very slight focal respiratory epithelial mineral deposit(s)

NO ABNORMALITIES DETECTED IN:
LARYNX

B0018 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Area of concern not included in section
LUNGS : Very slight large alveolar macrophages

NO ABNORMALITIES DETECTED IN:
LARYNX

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal Group:B 1mg/m3 Males

B0020 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LUNGS : Very slight large alveolar macrophages

NO ABNORMALITIES DETECTED IN:
LARYNX, NASAL CAVITY

B0022 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LUNGS : Very slight large alveolar macrophages
NASAL CAVITY : Very slight focal respiratory epithelial mineral deposit(s)

NO ABNORMALITIES DETECTED IN:
LARYNX

B0024 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Very slight mononuclear cell infiltrate
LUNGS : Very slight BALT germinal centre development
Very slight large alveolar macrophages

NO ABNORMALITIES DETECTED IN:
NASAL CAVITY

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal	Group:C	3mg/m3	Males

C0026 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Very slight mononuclear cell infiltrate
LUNGS : Very slight BALT germinal centre development
Slight large alveolar macrophages
NASAL CAVITY : Slight focal respiratory epithelial mineral deposit(s)

C0028 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Very slight focal squamous metaplasia
LUNGS : Very slight perivascular mononuclear cell infiltrate
Slight large alveolar macrophages
NASAL CAVITY : Slight focal respiratory epithelial mineral deposit(s)

C0030 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Slight mononuclear cell infiltrate
LUNGS : Slight perivascular mononuclear cell infiltrate
Very slight large alveolar macrophages
Very slight BALT germinal centre development
Slight bronchial/bronchiolar epithelial hypertrophy
NASAL CAVITY : Very slight focal olfactory epithelial necrosis , unilateral dorsal arch, level 4
Very slight focal respiratory epithelial mineral deposit(s)

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal	Group:C	3mg/m3	Males

C0032 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Lymphoid aggregates
Very slight mononuclear cell infiltrate
LUNGS : Very slight large alveolar macrophages
Very slight perivascular mononuclear cell infiltrate
Slight bronchial/bronchiolar epithelial hypertrophy

NO ABNORMALITIES DETECTED IN:
NASAL CAVITY

C0034 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Slight mononuclear cell infiltrate
Very slight focal squamous metaplasia
LUNGS : Slight bronchial/bronchiolar epithelial hypertrophy
Very slight perivascular mononuclear cell infiltrate
Slight BALT germinal centre development
Very slight focal alveolitis
Slight large alveolar macrophages
NASAL CAVITY : Slight focal olfactory epithelial vacuolation , level 6

C0036 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Lost at necropsy
LUNGS : Slight focal alveolitis
Slight large alveolar macrophages
NASAL CAVITY : Very slight focal respiratory epithelial mineral deposit(s)

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal	Group:D	10mg/m3	Males

D0038 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Very slight mononuclear cell infiltrate
Very slight focal squamous metaplasia
LUNGS : Very slight perivascular mononuclear cell infiltrate
Slight large alveolar macrophages
NASAL CAVITY : Slight focal respiratory epithelial mineral deposit(s)

D0040 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Very slight focal squamous metaplasia
LUNGS : Slight large alveolar macrophages
Very slight focal alveolitis

NO ABNORMALITIES DETECTED IN:
NASAL CAVITY

D0042 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Slight mononuclear cell infiltrate
Slight focal squamous metaplasia
LUNGS : Slight BALT germinal centre development
Moderate large alveolar macrophages
Slight perivascular mononuclear cell infiltrate
NASAL CAVITY : Slight focal respiratory epithelial mineral deposit(s)
Very slight goblet cell hyperplasia

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal Group:D 10mg/m3 Males

D0044 survivor
killed on day 28

Macroscopic findings
KIDNEYS : Uni-lateral hydronephrosis

Microscopic findings
LARYNX : Slight mononuclear cell infiltrate
LUNGS : Moderate perivascular mononuclear cell infiltrate
Slight BALT germinal centre development
Slight large alveolar macrophages
Slight bronchial/bronchiolar epithelial hypertrophy
Very slight focal alveolitis
NASAL CAVITY : Very slight focal olfactory epithelial vacuolation

D0046 survivor
killed on day 28

Macroscopic findings
TESTES : Uni-lateral small
Uni-lateral cryptorchism

Microscopic findings
LARYNX : Area of concern not included in section
LUNGS : Very slight perivascular mononuclear cell infiltrate
Slight large alveolar macrophages
Bone spherule/spicule
NASAL CAVITY : Moderate focal olfactory epithelial necrosis , level 6
Slight focal olfactory epithelial vacuolation , level 6
Slight focal respiratory epithelial mineral deposit(s)
Slight focal goblet cell hyperplasia , level 2

NO ABNORMALITIES DETECTED IN:
LARYNX

D0048 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Slight focal squamous metaplasia
Slight mononuclear cell infiltrate

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal Group:D 10mg/m3 Males

D0048 Continued....

Microscopic findings

LUNGS :	Slight BALT germinal centre development
	Slight large alveolar macrophages
	Slight perivascular mononuclear cell infiltrate
NASAL CAVITY	: Very slight focal respiratory epithelial mineral deposit(s)

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal	Group:E	100mg/m3	Males

E0050 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Slight focal squamous metaplasia
Slight focal subepithelial necrosis
Very slight focal subepithelial mineralisation
Focal granulomatous inflammation
LUNGS : Moderate large alveolar macrophages
Bone spherule/spicule
Moderate perivascular mononuclear cell infiltrate
Slight BALT germinal centre development
Very slight focal alveolitis
NASAL CAVITY : Moderate focal olfactory epithelial necrosis
Very slight focal respiratory epithelial mineral deposit(s)
Slight focal respiratory epithelial metaplasia
Very slight goblet cell hyperplasia

NO ABNORMALITIES DETECTED IN:
TRACHEA/BRONCHI

E0052 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Area of concern not included in section
LUNGS : Very slight focal alveolitis
Very slight perivascular mononuclear cell infiltrate
Moderate large alveolar macrophages
Alveolar proteinosis
Slight BALT germinal centre development
NASAL CAVITY : Moderate focal olfactory epithelial necrosis
Very slight focal respiratory epithelial mineral deposit(s)
Slight focal respiratory epithelial metaplasia
Very slight goblet cell hyperplasia

NO ABNORMALITIES DETECTED IN:
LARYNX, TRACHEA/BRONCHI

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal	Group:E	100mg/m3	Males

E0054 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Area of concern not included in section
LUNGS : Slight BALT germinal centre development
Very slight focal alveolitis
Moderate large alveolar macrophages
Slight perivascular mononuclear cell infiltrate
NASAL CAVITY : Moderate focal olfactory epithelial necrosis
Very slight focal respiratory epithelial mineral deposit(s)
Slight focal respiratory epithelial metaplasia
Very slight goblet cell hyperplasia

NO ABNORMALITIES DETECTED IN:
LARYNX, TRACHEA/BRONCHI

E0056 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Area of concern not included in section
Epithelial microcyst(s) , containing particulate matter
LUNGS : Alveolar proteinosis
Slight alveolitis
Slight perivascular mononuclear cell infiltrate
Slight BALT germinal centre development
Slight large alveolar macrophages
NASAL CAVITY : Moderate focal olfactory epithelial necrosis
Slight focal respiratory epithelial metaplasia
Very slight goblet cell hyperplasia
Very slight focal respiratory epithelial mineral deposit(s)

NO ABNORMALITIES DETECTED IN:
TRACHEA/BRONCHI

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal Group:E 100mg/m3 Males

E0058 survivor
killed on day 28

Macroscopic findings
THORACIC CAVITY : Enlarged parathymic lymphnodes

Microscopic findings
LARYNX : Slight focal squamous metaplasia
Focal granulomatous inflammation
LUNGS : Slight BALT germinal centre development
Moderate perivascular mononuclear cell infiltrate
Slight alveolitis
Slight bronchial/bronchiolar epithelial hypertrophy
Slight large alveolar macrophages
NASAL CAVITY : Slight focal olfactory epithelial necrosis
Slight focal respiratory epithelial metaplasia
Very slight goblet cell hyperplasia
Slight focal respiratory epithelial mineral deposit(s)
THORACIC CAVITY : Macrophage aggregate(s) parathymic lymphnodes

NO ABNORMALITIES DETECTED IN:
TRACHEA/BRONCHI

E0060 survivor
killed on day 28

MACROSCOPY : No gross lesions

Microscopic findings
LARYNX : Slight focal squamous metaplasia
Slight focal subepithelial necrosis
Focal granulomatous inflammation
Very slight focal subepithelial mineralisation
LUNGS : Alveolar proteinosis
Very slight perivascular mononuclear cell infiltrate
Slight alveolitis
Slight large alveolar macrophages
NASAL CAVITY : Moderate focal olfactory epithelial necrosis
Moderate respiratory epithelial metaplasia
Very slight goblet cell hyperplasia
Very slight focal respiratory epithelial mineral deposit(s)

NOCOLOK flux 28-day inhalation toxicology study in rats
TNO Nutrition and Food Research
Study: 4671/01
Appendix 7: Individual data pathology the day after the last exposure

Study:4671/01

Animal Group:E 100mg/m3 Males

E0060 Continued....

Microscopic findings
NO ABNORMALITIES DETECTED IN:
TRACHEA/BRONCHI

*** Listing Complete ***